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* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  JAN 27  Source of Registration (SR) information in REGISTRY updated
                  and searchable
 NEWS  4  JAN 27  A new search aid, the Company Name Thesaurus, available in
                  CA/CAplus
 NEWS  5  FEB 05  German (DE) application and patent publication number format
                  changes
 NEWS  6  MAR 03  MEDLINE and LMEDLINE reloaded
 NEWS  7  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  8  MAR 03  FRANCEPAT now available on STN
 NEWS  9  MAR 29  Pharmaceutical Substances (PS) now available on STN
 NEWS 10  MAR 29  WPIFV now available on STN
 NEWS 11  MAR 29  No connect hour charges in WPIFV until May 1, 2004
 NEWS 12  MAR 29  New monthly current-awareness alert (SDI) frequency in RAPRA
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 NEWS 14  APR 26  IFIPAT/IFIUDB/IFICDB: New super search and display field
                  available
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 NEWS 16  APR 27  NLDB: New search and display fields available

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               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
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FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 29 Apr 2004 (20040429/PD)
FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2004
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>>> the earliest to the latest publication. <<<

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COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 2.70 2.91

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 29 Apr 2004 (20040429/PD)
FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2004

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>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
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This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> e lynn ralf geiben/in
E1 1 LYNN PETER ROBERT/IN
E2 1 LYNN R KEITH/IN
E3 1 --> LYNN RALF GEIBEN/IN

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E5            4     LYNN RAY E/IN
E6            1     LYNN RICHARD BRIAN/IN
E7            2     LYNN RICKY L/IN
E8            1     LYNN ROBERT C/IN
E9            2     LYNN ROBERT E/IN
E10           2     LYNN ROBERT G/IN
E11           1     LYNN ROBERT J/IN
E12           1     LYNN ROBERT R/IN

=> s e3
L1            1 "LYNN RALF GEIBEN"/IN

=> d l1,cbib

L1   ANSWER 1 OF 1  USPATFULL on STN
2002:300810 Peroxiredoxin drugs for treatment of HIV-1 infection and methods of
    use thereof.
    Lynn, Ralf Geiben, Watertown, MA, UNITED STATES
    Walker, Bruce D., Milton, MA, UNITED STATES
    US 2002168353 A1 20021114
    APPLICATION: US 2002-57593  A1  20020125 (10)
    PRIORITY: US 2001-278234P  20010323 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d l1,cbib,ab,clm

L1   ANSWER 1 OF 1  USPATFULL on STN
2002:300810 Peroxiredoxin drugs for treatment of HIV-1 infection and methods of
    use thereof.
    Lynn, Ralf Geiben, Watertown, MA, UNITED STATES
    Walker, Bruce D., Milton, MA, UNITED STATES
    US 2002168353 A1 20021114
    APPLICATION: US 2002-57593  A1  20020125 (10)
    PRIORITY: US 2001-278234P  20010323 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      The invention includes compositions comprising substantially purified
        peroxiredoxin that are useful in methods for the treatment and
        prevention of HIV-1 infection. The invention also includes methods for
        the treatment and prevention of HIV-1 infection comprising contacting a
        composition of the invention with a human patient. Additionally, the
        invention includes antibodies and kits useful in the treatment and
        prevention of HIV-1 infection.
CLM     What is claimed is:
        1. A method of treating HIV-1 infection, the method comprising
        contacting a cell susceptible to HIV-1 infection with an amount of
        peroxiredoxin sufficient to inhibit infection of the cell by HIV-1.

        2. The method of claim 1, wherein the peroxiredoxin is selected from the
        group consisting of type I peroxiredoxin and type II peroxiredoxin.

        3. The method of claim 1, wherein the peroxiredoxin is
        protease-resistant.

        4. A method of decreasing the infectivity of HIV-1, if any is present,
        in a biological sample, the method comprising:  (a) identifying a
        biological sample in which a reduction or elimination of HIV-1
        infectivity is desirable; and  (b) contacting the biological sample with
        an amount of peroxiredoxin sufficient to decrease the infectivity of
        HIV-1 in the biological sample.

        5. The method of claim 3, wherein the biological sample is selected from
        the group consisting of: blood, plasma, serum, saliva, semen, cervical
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[illegible], amniotic fluids.

6. The method of claim 3, wherein the peroxiredoxin is selected from the group consisting of: type I peroxiredoxin and type II peroxiredoxin.

7. The method of claim 3, wherein the peroxiredoxin is protease-resistant.

8. The method of claim 3, wherein the amount of peroxiredoxin is at least about 5 μg/ml of the biological sample volume.

9. The method of claim 3 wherein the amount of peroxiredoxin is at least about 10 μg/ml of the biological sample volume.

10. A method of treating HIV-1 infection, the method comprising contacting a cell susceptible to HIV-1 infection with an amount of manganese dismutase sufficient to inhibit infection of the cell by HIV-1.

11. A method of treating HIV-1 infection, the method comprising introducing into a cell susceptible to HIV-1 infection a DNA molecule encoding a peroxiredoxin, and expressing the peroxiredoxin in an amount sufficient to inhibit infection of the cell by the HIV-1.

12. A method of treating HIV-1 infection in a subject, the method comprising introducing into the subject a cell that expresses a peroxiredoxin in an amount sufficient to inhibit infection of an endogenous cell of the subject, the endogenous cell being susceptible to HIV-1 infection.

13. A biological sample purification system to reduce the number of HIV-1 particles in a biological sample, comprising a peroxiredoxin linked to a surface.

14. A biological sample purification system to reduce the number of HIV-1 particles in a biological sample, comprising a peroxiredoxin linked to a surface, wherein contacting said biological sample and said biological sample purification system results in a reduction in the number of HIV-1 particles present in the biological sample.

15. The purification system of claim 14, wherein said surface is a bead, chip, column, or matrix.

16. A pharmaceutical composition for the treatment or prevention of HIV infection in a subject, comprising a peroxiredoxin and a pharmaceutically acceptable carrier.

17. A kit comprising in one or more containers the pharmaceutical composition of claim 16.

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=> e walker bruce d/in
E1          1     WALKER BRUCE A/IN
E2          1     WALKER BRUCE CLARKE/IN
E3          7 --> WALKER BRUCE D/IN
E4          2     WALKER BRUCE E/IN
E5          1     WALKER BRUCE G/IN
E6          6     WALKER BRUCE H/IN
E7          2     WALKER BRUCE HUNTRESS/IN
E8          5     WALKER BRUCE J/IN
E9          3     WALKER BRUCE K/IN
E10         3     WALKER BRUCE R/IN
E11         1     WALKER BRUCE W/IN
E12         2     WALKER BRYAN J/IN
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L2            7 "WALKER BRUCE D"/IN

=> s l2 not l1
L3            6 L2 NOT L1

=> d l3,ti,1-6

L3   ANSWER 1 OF 6  USPATFULL on STN
TI     Cytotoxic T-cell epitopes of HIV-1 virus

L3   ANSWER 2 OF 6  USPATFULL on STN
TI     Epitopes of human immunodeficiency virus-1

L3   ANSWER 3 OF 6  USPATFULL on STN
TI     Therapeutic anti-HIV (vpr) compounds

L3   ANSWER 4 OF 6  USPATFULL on STN
TI     Therapeutic anti-HIV (IV9) compounds

L3   ANSWER 5 OF 6  USPATFULL on STN
TI     Serpin drugs for treatment of HIV infection and method of use thereof

L3   ANSWER 6 OF 6  USPATFULL on STN
TI     Method of eliciting anti-HIV-1 helper T cell responses


=> d l3,cbib,ab,1-6

L3   ANSWER 1 OF 6  USPATFULL on STN
2004:1826 Cytotoxic T-cell epitopes of HIV-1 virus.
    Altfeld, Marcus, Arlington, MA, UNITED STATES
    Yu, Xu, Cambridge, MA, UNITED STATES
    Walker, Bruce D., Milton, MA, UNITED STATES
    Addo, Marylyn, Arlington, MA, UNITED STATES
    US 2004001845 A1 20040101
    APPLICATION: US 2003-442909  A1  20030520 (10)
    PRIORITY: US 2002-382120P  20020520 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention provides compositions containing HIV epitopes, which are
       recognized by cytotoxic T lymphocytes (CTL). Such polypeptides are used
       in vaccines and immunotherapies. HIV-1 epitopes represent early targets
       in a naturally-occurring response against HIV-1 infection.

L3   ANSWER 2 OF 6  USPATFULL on STN
2003:311855 Epitopes of human immunodeficiency virus-1.
    Walker, Bruce D., Milton, MA, UNITED STATES
    Altfeld, Marcus, Arlington, MA, UNITED STATES
    US 2003219450 A1 20031127
    APPLICATION: US 2002-222463  A1  20020816 (10)
    PRIORITY: US 2001-313408P  20010816 (60)
    US 2001-313208P  20010817 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention features an immunogenic composition containing a
       frequently-recognized epitope of an HIV-1 accessory protein and methods
       of inducing an immune response using such an epitope. The epitope
       peptide contains an amino acid sequence of a functionally active domain
       or a structural domain of the accessory protein.

L3   ANSWER 3 OF 6  USPATFULL on STN
2003:237360 Therapeutic anti-HIV (vpr) compounds.
    Nicolette, Charles A., Framingham, MA, UNITED STATES
    Walker, Bruce D., Milton, MA, UNITED STATES
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APPLICATION: US 2002-283618 A1 20021029 (10)
PRIORITY: US 2001-345957P 20011029 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides synthetic compounds, antibodies that recognize and bind to these compounds, polynucleotides that encode these compounds, and immune effect or cells raised in response to presentation of these epitopes. The invention further provides methods for inducing an immune response and administering immunotherapy to a subject by delivering the compositions of the invention.

L3 ANSWER 4 OF 6 USPATFULL on STN
2003:232519 Therapeutic anti-HIV (IV9) compounds.
Nicolette, Charles A., Framingham, MA, UNITED STATES
Walker, Bruce D., Milton, MA, UNITED STATES
US 2003162720 A1 20030828
APPLICATION: US 2002-283847 A1 20021029 (10)
PRIORITY: US 2001-345116P 20011029 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides synthetic compounds, antibodies that recognize and bind to these compounds, polynucleotides that encode these compounds, and immune effector cells raised in response to presentation of these epitopes. The invention further provides methods for inducing an immune response and administering immunotherapy to a subject by delivering the compositions of the invention.

L3 ANSWER 5 OF 6 USPATFULL on STN
2002:235509 Serpin drugs for treatment of HIV infection and method of use thereof.
Geiben Lynn, Ralf, Watertown, MA, UNITED STATES
Walker, Bruce D., Milton, MA, UNITED STATES
US 2002127698 A1 20020912
APPLICATION: US 2002-57613 A1 20020125 (10)
PRIORITY: US 2001-264338P 20010126 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention includes compositions comprising substantially purified serpin that are useful in methods for the treatment and prevention of HIV infection. The invention also includes methods for the treatment and prevention of HIV infection comprising contacting a composition of the invention with a human patient or treating HIV infection by introducing into a cell susceptible to HIV infection a DNA molecule encoding a serpin. Additionally, the invention includes antibodies and kits useful in the detection, treatment, and prevention of HIV infection.

L3 ANSWER 6 OF 6 USPATFULL on STN
1999:132231 Method of eliciting anti-HIV-1 helper T cell responses.
Walker, Bruce D., Milton, MA, United States
The General Hospital Corporation, Boston, MA, United States (U.S. corporation)
US 5972339 19991026
APPLICATION: US 1997-969721 19971113 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method of producing an HIV-specific helper T cell response in an animal by (1) providing a polypeptide 8 to 50 amino acid residues in length and having a helper T cell epitope of a HIV-1 p24 peptide; and (2) administering to the animal an amount of the polypeptide sufficient to produce an HIV-specific helper T cell response.

=> d l3,cbib,ab,clm,3-5

L3 ANSWER 3 OF 6 USPATFULL on STN

2003:232519 Therapeutic anti-HIV (IV9) compounds.
Nicolette, Charles A., Framingham, MA, UNITED STATES
Walker, Bruce D., Milton, MA, UNITED STATES
US 2003165517 A1 20030904
APPLICATION: US 2002-283618 A1 20021029 (10)
PRIORITY: US 2001-345957P 20011029 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides synthetic compounds, antibodies that recognize and bind to these compounds, polynucleotides that encode these compounds, and immune effect or cells raised in response to presentation of these epitopes. The invention further provides methods for inducing an immune response and administering immunotherapy to a subject by delivering the compositions of the invention.
CLM What is claimed is:
1. A composition comprising at least one immunogenic ligand, wherein said immunogenic ligand is individually characterized by an ability to elicit an immune response against the same native ligand, and wherein said immunogenic ligand is selected from FLYEQGMFV (SEQ ID NO:1); FLYEQGIFV (SEQ ID NO:3); FLKMWKDAV (SEQ ID NO:5); FLSWTLPRV (SEQ ID NO:7); FLGGHWGTV (SEQ ID NO:9); and FLWWFTSTV (SEQ ID NO:11).

2. The composition of claim 1, further comprising a carrier.

3. The composition of claim 2, wherein the carrier is a pharmaceutically acceptable carrier.

4. A host cell comprising the composition of claim 1.

5. The host cell of claim 4, wherein the host cell is an antigen presenting cell and the immunogenic ligands are presented on the surface of the cell.

6. The host cell of claim 5, wherein the antigen presenting cell is a dendritic cell.

7. A composition comprising the host cell of any one of claims 4 to 6 and a carrier.

8. The composition of claim 7, wherein the carrier is a pharmaceutically acceptable carrier.

9. A method for inducing an immune response in a subject, comprising delivering to the subject comprising delivering an effective amount of the composition of claim 1.

L3 ANSWER 4 OF 6 USPATFULL on STN
2003:232519 Therapeutic anti-HIV (IV9) compounds.
Nicolette, Charles A., Framingham, MA, UNITED STATES
Walker, Bruce D., Milton, MA, UNITED STATES
US 2003162720 A1 20030828
APPLICATION: US 2002-283847 A1 20021029 (10)
PRIORITY: US 2001-345116P 20011029 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides synthetic compounds, antibodies that recognize and bind to these compounds, polynucleotides that encode these compounds, and immune effector cells raised in response to presentation of these epitopes. The invention further provides methods for inducing an immune response and administering immunotherapy to a subject by delivering the compositions of the invention.
CLM What is claimed is:
1. A composition comprising at least one immunogenic ligand, wherein said immunogenic ligand is individually characterized by an ability to elicit an immune response against the same native ligand, and wherein

said immunogenic ligand is selected from selected from the group consisting of FLEMHAYLV (SEQ ID NO:1); FLEKHAYIV (SEQ ID NO:3); FLNAARRVV (SEQ ID NO:5); FLIGRTLXV (SEQ ID NO:7); FLYTVDVPV (SEQ ID NO:9); FLWYPVYXV (SEQ ID NO:11); and FLYQMKIA (SEQ ID NO: 13) and wherein X is any amino acid.

2. The composition of claim 1, further comprising a carrier.

3. The composition of claim 2, wherein the carrier is a pharmaceutically acceptable carrier.

4. A host cell comprising the composition of claim 1.

5. The host cell of claim 4, wherein the host cell is an antigen presenting cell and the immunogenic ligands are presented on the surface of the cell.

6. The host cell of claim 5, wherein the antigen presenting cell is a dendritic cell.

7. A composition comprising the host cell of any one of claims 4 to 6 and a carrier.

8. The composition of claim 7, wherein the carrier is a pharmaceutically acceptable carrier.

9. A method for inducing an immune response in a subject, comprising delivering to the subject comprising delivering an effective amount of the composition of claim 1.

L3 ANSWER 5 OF 6 USPATFULL on STN

2002:235509 Serpin drugs for treatment of HIV infection and method of use thereof.

Geiben Lynn, Ralf, Watertown, MA, UNITED STATES

Walker, Bruce D., Milton, MA, UNITED STATES

US 2002127698 A1 20020912

APPLICATION: US 2002-57613 A1 20020125 (10)

PRIORITY: US 2001-264338P 20010126 (60)

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention includes compositions comprising substantially purified serpin that are useful in methods for the treatment and prevention of HIV infection. The invention also includes methods for the treatment and prevention of HIV infection comprising contacting a composition of the invention with a human patient or treating HIV infection by introducing into a cell susceptible to HIV infection a DNA molecule encoding a serpin. Additionally, the invention includes antibodies and kits useful in the detection, treatment, and prevention of HIV infection.

CLM What is claimed is:

1. A method of inhibiting the infectivity of HIV, said method comprising the steps of: (a) contacting an HIV virion with a composition comprising a serpin, or a analog thereof; and (b) incubating said virion with said serpin, or analog thereof, for a period of time sufficient to inhibit the infectivity of HIV.

2. The method of claim 1, wherein said serpin has the following characteristics: (i) inhibits serine protease; and (ii) binds heparin.

3. The method of claim 1, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitryp sin.

4. The method of claim 1, wherein said serpin is bovine-originated or human-originated.

5. The method of claim 1, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

6. The method of claim 1, further comprising pretreating said serpin before contacting the serpin with the HIV vinon.

7. The method of claim 6, wherein said pretreatment is contacting the serpin with elastase.

8. A method of decreasing the infectivity of HIV, if any is present, in a biological sample, the method comprising the steps of: (a) identifying a biological sample in which a decrease or elimination of HIV infectivity is desirable; and (b) contacting the biological sample with an amount of serpin, or analog thereof, sufficient to decrease the infectivity of HIV in the biological sample.

9. The method of claim 8, wherein said serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

10. The method of claim 8, wherein said biological sample is selected from a group consisting of: blood, plasma, serum, saliva, semen, cervical secretions, urine, breast milk, and amniotic fluids.

11. The method of claim 8, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitrypsin.

12. The method of claim 8, wherein said serpin is bovine-originated or human-originated.

13. The method of claim 8, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

14. The method of claim 8, further comprising pretreating said serpin before contacting the serpin with the biological sample.

15. The method of claim 14, wherein the pretreatment is contacting said serpin with elastase.

16. The method of claim 8, wherein the amount of said serpin is at least about 2 units per milliliter of the biological sample volume.

17. The method of claim 8, wherein the amount of said serpin is at least about 5 units per milliliter of the biological sample volume.

18. The method of claim 8, wherein the amount of said serpin is at least about 10 units per milliliter of the biological sample volume.

19. A method of treating HIV infection, the method comprising introducing into a cell susceptible to HIV infection a DNA molecule encoding a serpin, or analog thereof, and expressing said serpin, or analog thereof, in an amount sufficient to inhibit infection of the cell by the HIV.

20. The method of claim 19, wherein said DNA encodes a serpin selected from a group consisting of: antithrombin, protein C-inhibitor, plasminogen activator inhibitor, activated protein C, and alpha-1-antitrypsin.

21. The method of claim 19, wherein the expressed serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

22. The method of claim 19, wherein said DNA encodes a serpin that is

bovine-originated or human-originated.

23. The method of claim 19, wherein the expressed serpin is selected from the group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

24. A method of treating HIV infection in a subject, the method comprising introducing into the subject a producer cell that expresses a serpin, or analog thereof, in an amount sufficient to inhibit infection of an endogenous cell of the subject, the endogenous cell being susceptible to HIV infection.

25. The method of claim 24, wherein said serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

26. The method of claim 24, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitrypsin.

27. The method of claim 24, wherein said serpin is bovine-originated or human-originated.

28. The method of claim 24, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

29. A composition comprising a serpin, or analog thereof, associated with a surface.

30. The composition of claim 29, wherein said serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

31. The composition of claim 29, wherein said surface is a bead, chip, column, or matrix.

32. The composition of claim 29, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitrypsin.

33. The composition of claim 29, wherein said serpin is bovine-originated or human-originated.

34. The composition of claim 29, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

35. A method of inhibiting the infectivity of HIV, said method comprising the steps of: (a) contacting an HIV virion with a composition having a surface which comprises substantially purified serpin, or analog thereof, associated with said surface; and (b) incubating said HIV virion with said serpin for a period of time sufficient to inhibit the infectivity of HIV.

36. The method of claim 35, wherein said surface is the composition of claim 29.

37. A pharmaceutical composition comprising in a therapeutically effective amount of a serpin, or analog thereof, to inhibit, to treat, or prevent HIV-infection, and a pharmaceutically acceptable carrier.

38. The composition of claim 37, wherein said serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

39. The composition of claim 37, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitrypsin.

40. The composition of claim 37, wherein said serpin is bovine-originated or human-originated.

41. The composition of claim 37, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

42. A kit comprising, in one or more containers, the pharmaceutical composition of claim 37.

43. A kit for detecting a protein which inhibits the infectivity of HIV, said kit comprising an antibody which specifically binds a serpin, or analog thereof.

44. The kit of claim 43, wherein said serpin has the following characteristics: (a) inhibits serine protease; and (b) binds heparin.

45. The kit of claim 43, wherein said serpin is selected from a group consisting of: antithrombin, protein C-inhibitor, activated protein C, plasminogen activator inhibitor, and alpha-1-antitrypsin.

46. The kit of claim 43, wherein said serpin is bovine-originated or human-originated.

47. The kit of claim 43, wherein said serpin is selected from a group consisting of: a 43 kDa modified antithrombin; R-antithrombin; S-antithrombin; or a combination thereof.

48. The kit of claim 43, wherein said detection reagent is selected from the group consisting of an enzyme and a radionuclide.

=> file wpids
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	29.75	32.66

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
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FILE LAST UPDATED: 29 APR 2004 <20040429/UP>
MOST RECENT DERWENT UPDATE: 200428 <200428/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE, PLEASE VISIT:
http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE http://thomsonderwent.com/coverage/latestupdates/ <<<

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>>> NEW! FAST-ALERTING ACCESS TO NEWLY-PUBLISHED PATENT DOCUMENTATION NOW AVAILABLE IN DERWENT WORLD PATENTS INDEX FIRST VIEW - FILE WPIFV. FREE CONNECT HOUR UNTIL 1 MAY 2004. FOR FURTHER DETAILS: http://www.thomsonderwent.com/dwpifv <<<

>>> NEW! IMPROVE YOUR LITIGATION CHECKING AND INFRINGEMENT MONITORING WITH LITALERT. FIRST ACCESS TO RECORDS OF IP LAWSUITS FILED IN THE 94 US DISTRICT COURTS SINCE 1973. FOR FURTHER DETAILS:

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>>> THE DISPLAY LAYOUT HAS BEEN CHANGED TO ACCOMODATE THE
    NEW FORMAT GERMAN PATENT APPLICATION AND PUBLICATION
    NUMBERS. SEE ALSO:
    http://www.stn-international.de/archive/stnews/news0104.pdf <<<

>>> SINCE THE FILE HAD NOT BEEN UPDATED BETWEEN APRIL 12-16
    THERE WAS NO WEEKLY SDI RUN  <<<

=> e lynn r g/in
E1            1     LYNN R C/IN
E2            2     LYNN R E/IN
E3            2 --> LYNN R G/IN
E4            1     LYNN R K/IN
E5            2     LYNN R L/IN
E6            1     LYNN R R/IN
E7           18     LYNN R W/IN
E8           19     LYNN S/IN
E9            4     LYNN S A/IN
E10           2     LYNN S J/IN
E11          10     LYNN S R/IN
E12           2     LYNN S S/IN

=> s e3
L4            2 "LYNN R G"/IN

=> d l4,bib,1-2

L4   ANSWER 1 OF 2  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN   2003-018949 [01]   WPIDS
DNC  C2003-004716
TI   Treating human immunodeficiency virus-1 infection by contacting cell
     susceptible to HIV-1 infection with peroxiredoxin, or introducing into
     cell susceptible to HIV infection a DNA molecule encoding peroxiredoxin.
DC   B04 B07 D16
IN   LYNN, R G; WALKER, B D
PA   (LYNN-I) LYNN R G; (WALK-I) WALKER B D; (GEHO) GEN HOSPITAL CORP
CYC  101
PI   WO 2002077294   A1 20021003 (200301)* EN   47
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            NL OA PT SD SE SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2002168353   A1 20021114 (200301)
     EP 1370697      A1 20031217 (200402)  EN
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI TR
ADT  WO 2002077294 A1 WO 2002-US8077 20020318; US 2002168353 A1 Provisional US
     2001-278234P 20010323, US 2002-57593 20020125; EP 1370697 A1 EP
     2002-731136 20020318, WO 2002-US8077 20020318
FDT  EP 1370697 A1 Based on WO 2002077294
PRAI US 2002-57593        20020125; US 2001-278234P      20010323

L4   ANSWER 2 OF 2  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN   2002-627378 [67]   WPIDS
DNC  C2004-012671
TI   Composition useful for treating HIV infections and decreasing the
     infectivity of biological samples comprises a serine protease inhibiting
     serpin or its analog.
DC   B04
IN   LYNN, R G; WALKER, B D; GEIBEN LYNN, R
```

CYC 99
PI WO 2002058638 A2 20020801 (200267)* EN 47
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO
RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
US 2002127698 A1 20020912 (200267)
EP 1362127 A2 20031119 (200377) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI TR
AU 2002248384 A1 20020806 (200427)
ADT WO 2002058638 A2 WO 2002-US2309 20020125; US 2002127698 A1 Provisional US
2001-264338P 20010126, US 2002-57613 20020125; EP 1362127 A2 EP
2002-717374 20020125, WO 2002-US2309 20020125; AU 2002248384 A1 AU
2002-248384 20020125
FDT EP 1362127 A2 Based on WO 2002058638; AU 2002248384 A1 Based on WO
2002058638
PRAI US 2001-264338P 20010126; US 2002-57613 20020125

=> d l4,bib,ab,clm,1-2
'CLM' IS NOT A VALID FORMAT FOR FILE 'WPIDS'

The following are valid formats:

TRI	SAM	Short Information (Syn.: TRIAL,SAMPLE)
STR		DERWENT Chemical Resource Structure
BIB		Bibliographic Data
BRIEFG.H		Brief Contents of Document with GI.H
BRIEFG		Brief Contents of Document with GI
BRIEF		Brief Contents of Document
IBRIEFG.H		Brief Contents of Document with GI.H, Indented Version
IBRIEFG		Brief Contents of Document with GI, Indented Version
IBRIEF		Brief Contents of Document, Indented Version
MAXG		All Data with GIS and GI.H
MAX		All Data
ALLG.H		All Data Except ABEQ, CMC, and PLC with GI.H
ALLG		All Data Except ABEQ, CMC, and PLC with GI
ALL		All Data Except ABEQ, CMC, and PLC
FULL		All Data Except ABEQ, CMC, and PLC plus TECH and PRIO
FULLG		All Data Except ABEQ, CMC, and PLC with GI plus TECH and PRIO
DALL		Delimited ALL Format
BASIC		Basic Patent Information
STD		Default
IDE		Structure File Default
IALLG.H		Indented Version of ALL Format with GI.H
IALLG		Indented Version of ALL Format with GI
IALL		Indented Version of ALL Format
IFULL		Indented Version of FULL Format
IFULLG		Indented Version of FULLG Format
ISTD		Indented Version of STD Format
IBIB		Indented Version of BIB Format
ABS		All Abstracts
CODE	IND	Manual-, Plasdoc-, and Chemical Code plus Keywords
SUM		Title and Novelty

AB		Abstract (Basic)
ABEQ		Abstract, Equivalent
ADT		Application Details
ADT.B		Application Details Basic
AI	AP	Application Information
AI.B		Application Information Basic

AN		Accession Number
AN.S		DERWENT Chemistry Resource Accession Number, DCR Segment
APPS		Application Number Group
AW		Additional Words
CC		Classification Code (Substance Descriptor
CMC		Chemical Code
CMT		Comment
CN		Chemical Name
CN.P		Chemical Name Preferred
CN.S		Systematic Chemical Name
CR	XR	Cross Reference
CYC		Country Count
DAN		DERWENT Accession Number List
DC		DERWENT Class
DCN		DERWENT Compound Number
DCR		DERWENT Chemistry Resource Accession Number
DCRE		DERWENT Chemistry Resource Number
DCSE		DERWENT Chemistry Resource Number, DCR Segment
DN		Document Number CPI and Non CPI
DNC		Document Number CPI
DNN		Document Number Non CPI
DRN		DERWENT Registry Number
DS		Designated States
ED		Entry Date
EDCR		Entry Date DERWENT Chemistry Resource
FA		Field Availability
FAS		Field Availability Supplementary Data
FAM		Patent Family
FDT		Filing Details
FG	AM	Fragment Code
FS		File Segment
IC		International Patent Classification
GI		Graphical Information
GI.H		Graphical Information, High Resolution
GIS		Graphical Information Size
ICA		IPC, Additional (Supplementary)
ICI		IPC, Index (Complementary)
ICM		IPC, Main
ICS		IPC, Secondary
IN	AU	Inventor
IPC		International Patent Classification
ISMI		ISOSMILES String
KS		Plasdoc Key Serials
KW		Keyword Indexing, Including DERWENT Chemistry Resource Numbers, DWPI Segment
M0		Chemical Code (Pre 1970)
M1-6		Chemical Codes
MC		Manual Code
MF		Molecular Formula
MW		Molecular Weight
NOV		Novelty
PA	CS	Patent Assignee
PATS		Patent Number Group
PI	PN	Patent Information
PI.B	PN.B	Patent Information Basic
PIA		Patent Information Abbreviated
PIA.B		Patent Information Abbreviated Basic
PLC		Plasdoc Codes
PLE		Enhanced Plasdoc Codes
PNC		Patent Number Count
PRAI	PRN	Priority Information
PRIO		Prior Art
REP	RPN RE	Reference Patent Information
RIN		Ring Index Number
SDCN		Structure Segment DERWENT Compound Number
SDRN		Structure Segment DERWENT Registry Number
SMF		Standardized Molecular Formula

SRIN Structure Segment Ring Index Number
SY Synonym Name
TECH Technology Focus
TI Title
TT Title Terms
UP Update Date
UPA Update Date Plasdoc Code
UPAB Update Date Abstract
UPB Update Date Chemical Code
UPCR Update Date DERWENT Chemistry Resource
UPKW Update Date Keyword Indexing
UPP Update Date Patent
UPS Update Date SDI
UPTX Update New Content Abstract Fields
UPWX Update Date WPI Cross Reference
ENTER DISPLAY FORMAT (STD):bib,ab

L4 ANSWER 1 OF 2 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-018949 [01] WPIDS
DNC C2003-004716
TI Treating human immunodeficiency virus-1 infection by contacting cell susceptible to HIV-1 infection with peroxiredoxin, or introducing into cell susceptible to HIV infection a DNA molecule encoding peroxiredoxin.
DC B04 B07 D16
IN **LYNN, R G**; WALKER, B D
PA (LYNN-I) LYNN R G; (WALK-I) WALKER B D; (GEHO) GEN HOSPITAL CORP
CYC 101
PI WO 2002077294 A1 20021003 (200301)* EN 47
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
US 2002168353 A1 20021114 (200301)
EP 1370697 A1 20031217 (200402) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
ADT WO 2002077294 A1 WO 2002-US8077 20020318; US 2002168353 A1 Provisional US 2001-278234P 20010323, US 2002-57593 20020125; EP 1370697 A1 EP 2002-731136 20020318, WO 2002-US8077 20020318
FDT EP 1370697 A1 Based on WO 2002077294
PRAI US 2002-57593 20020125; US 2001-278234P 20010323
AB WO 200277294 A UPAB: 20030101
NOVELTY - Treating human immunodeficiency virus-1 (HIV-1) infection, is new

DETAILED DESCRIPTION - Treating:

(a) human immunodeficiency virus-1 (HIV-1) infection involves contacting a cell susceptible to HIV-1 infection with peroxiredoxin (I), or introducing into a cell susceptible to HIV infection a DNA molecule encoding (I), and expressing (I), to inhibit infection of cell by HIV-1;

(b) HIV-1 infection in a subject involves introducing into the subject cell expressing (I) to inhibit infection of an endogenous cell of the subject, the endogenous cell being susceptible to HIV-1 infection.

INDEPENDENT CLAIMS are also included for the following:

(1) decreasing (M1) infectivity of HIV-1, if any is present, in a biological sample, involves identifying a biological sample in which a reduction or elimination of HIV-1 infectivity is desirable, and contacting the sample with (I) to decrease the infectivity of HIV-1 in the sample;

(2) treating HIV-1 infection by contacting cell susceptible to HIV-1 infection with manganese dismutase sufficient to inhibit infection of the cell by HIV-1;

(3) a biological sample purification system (II) to reduce the number of HIV-1 particles in a biological sample comprising (I) linked to a

reduction in the number of HIV-1 particles present in the biological sample;

(4) a pharmaceutical composition (III) for the treatment or prevention of HIV-1 infection in a subject comprising (I) and a carrier; and

(5) a kit comprising (III) in one or more containers.

ACTIVITY - Anti-HIV.

H9 or PM1 cell-bearing polyvinylidene fluoride fibers (500000 MW cutoff) were prepared by filling conditioned hollow fibers with cell inoculum (uninfected cells, acutely HIV infected cells or chronically HIV infected cells) (Hollingshed et al., LIFE SCI., 57:131-41 (1995)). These inoculated hollow fibers were surgically implanted either subcutaneously or in the peritoneal cavity of severe combined immunodeficiency (SCID) mice (SCID/NCr). Hollow-fiber-bearing SCID mice were dosed either acutely or chronically with increasing amounts of purified peroxiredoxin preparation. The peroxiredoxin preparation (3-500 micro g/mouse/day) was administered to the hollow-fiber-bearing SCID mice by subcutaneous injection, intraperitoneal injection, intravenous or oral routes. At select times, blood was sampled from control and test animals and serum prepared. The amount of viral particles in test and control serum was measured by p24 enzyme linked immunosorbent assay (ELISA). Peroxiredoxin antiviral action yielded a significant decrease in viral load, as judged by a 15% decrease in serum p24 protein content in peroxiredoxin-treated animals relative to the serum p24 content of the untreated control animals.

MECHANISM OF ACTION - Inhibitor of HIV-1 infection of the cell.

USE - For treating HIV-1 infection in a cell or in a subject. (M1) is useful for decreasing infectivity of HIV-1, if any is present, in a biological sample such as blood, plasma, serum, saliva, semen, cervical secretions, saliva, urine, breast milk, cell culture medium or amniotic fluids. (III) is useful for treating or preventing HIV-1 infection in a subject (all claimed). The method is thus useful for treating acquired immunodeficiency syndrome (AIDS) in a mammal, preferably human. (M1) is useful for inhibiting infectivity of HIV-1 in biological fluids, e.g., in a hospital setting, where medical personnel are exposed to infectious HIV-1 secretions.

Dwg.0/7

L4 ANSWER 2 OF 2 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-627378 [67] WPIDS
DNC C2004-012671
TI Composition useful for treating HIV infections and decreasing the infectivity of biological samples comprises a serine protease inhibiting serpin or its analog.
DC B04
IN **LYNN, R G**; WALKER, B D; GEIBEN LYNN, R
PA (GEHO) GEN HOSPITAL CORP; (LYNN-I) GEIBEN LYNN R; (WALK-I) WALKER B D
CYC 99
PI WO 2002058638 A2 20020801 (200267)* EN 47
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
US 2002127698 A1 20020912 (200267)
EP 1362127 A2 20031119 (200377) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
AU 2002248384 A1 20020806 (200427)
ADT WO 2002058638 A2 WO 2002-US2309 20020125; US 2002127698 A1 Provisional US 2001-264338P 20010126, US 2002-57613 20020125; EP 1362127 A2 EP 2002-717374 20020125, WO 2002-US2309 20020125; AU 2002248384 A1 AU 2002-248384 20020125

2002058638
PRAI US 2001-264338P 20010126; US 2002-57613 20020125
AB WO 200258638 A UPAB: 20040426
NOVELTY - A pharmaceutical composition (C1) comprises serpin or its analog and a carrier.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) Inhibiting HIV infectivity involving contacting and then incubating an HIV virion with (C2);

(2) A kit (k1) comprising (C1) in at least one container;

(3) A kit (k2) for detecting a protein which inhibits HIV infectivity, comprising an antibody which specifically binds a serpin or its analog;

(4) Decreasing HIV infectivity in a biological sample involving identifying the biological sample and contacting it with serpin or its analog; and

(5) Treating HIV infection involving introducing a DNA molecule encoding a serpin or its analog and expressing the serpin or its analog, or introducing into a subject a producer cell that expresses serpin or its analog.

ACTIVITY - Anti-HIV.

H9 or PM1 cell-bearing polyvinylidene fluoride fibers (molecular weight = 500,000, internal diameter = 1 mm)were prepared by filling conditioned hollow fibers with cell inoculum (uninfected cells, acutely HIV infected cells or chronically HIV infected cells). These inoculated hollow fibers were surgically implanted either subcutaneously or in the peritoneal cavity of SCID mice. The hollow-fiber-bearing SCID mice were then dosed either acutely or chronically with increasing amounts of purified antithrombin (ATIII) preparation. The ATIII preparation (3 - 500 U) was administered to the mice by subcutaneous injection, intraperitoneal injection, intravenous or oral routes. At select times, blood was sampled from control and test animals and serum was prepared. The amount of viral particles in the test and control serum was measured. The ATIII-mediated antiviral action yielded a significant decrease of at least 15 % in serum p24 protein content in ATIII-treated animals as compared to that of the untreated control animals.

MECHANISM OF ACTION - Serine protease inhibitor; Heparin binder; HIV infectivity inhibitor.

USE - For inhibiting and treating HIV infectivity and for decreasing HIV infectivity in biological samples e.g. blood, plasma, serum, saliva, semen, cervical secretions, urine, breast milk and amniotic fluids (all claimed).

ADVANTAGE - The serpin inhibits serine protease and binds heparin.

Dwg.0/4

=> file medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	24.90	57.56

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004

FILE LAST UPDATED: 1 MAY 2004 (20040501/UP). FILE COVERS 1951 TO DATE.

On February 29, 2004, the 2004 MeSH terms were loaded. See HELP RLOAD for details. OLDMEDLINE now back to 1951.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a description of changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

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E1            8      LYNN R C/AU
E2            1      LYNN R D/AU
E3            0 --> LYNN R G/AU
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E12           1      LYNN RANDY C/AU

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E7            2      LYNN ROBERT/AU
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E9           41      LYNN S/AU
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E11           5      LYNN S C JR/AU
E12           2      LYNN S D/AU

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E9            1      GEIBINGER C/AU
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E11           2      GEIC M/AU
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E4            3      GEIBEN LYNN RALF/AU
E5            1      GEIBEN R/AU
E6            1      GEIBERGER K/AU
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E8            1      GEIBERT R C/AU
E9            1      GEIBETALER SVEN/AU
E10           6      GEIBIG C B/AU
E11           1      GEIBINGER C/AU
E12           3      GEIBULLAEV A A/AU

=> s e3 or e4
              2 "GEIBEN LYNN R"/AU
              3 "GEIBEN LYNN RALF"/AU
L5            5 "GEIBEN LYNN R"/AU OR "GEIBEN LYNN RALF"/AU

=> d l5,ti,1-5

L5   ANSWER 1 OF 5     MEDLINE on STN
TI   HIV-1 antiviral activity of recombinant natural killer cell enhancing
```

factors, NKEF-A and NKEF-B, members of the peroxiredoxin family.

L5 ANSWER 2 OF 5 MEDLINE on STN
TI Anti-human immunodeficiency virus noncytolytic CD8+ T-cell response: a review.

L5 ANSWER 3 OF 5 MEDLINE on STN
TI Purification of a modified form of bovine antithrombin III as an HIV-1 CD8+ T-cell antiviral factor.

L5 ANSWER 4 OF 5 MEDLINE on STN
TI Flagellin inhibits Myoviridae phage phiCTX infection of Pseudomonas aeruginosa strain GuA18: purification and mapping of binding site.

L5 ANSWER 5 OF 5 MEDLINE on STN
TI Noncytolytic inhibition of X4 virus by bulk CD8(+) cells from human immunodeficiency virus type 1 (HIV-1)-infected persons and HIV-1-specific cytotoxic T lymphocytes is not mediated by beta-chemokines.

=> d 15,cbib,ab,1-3,5

L5 ANSWER 1 OF 5 MEDLINE on STN
2003032310. PubMed ID: 12421812. HIV-1 antiviral activity of recombinant natural killer cell enhancing factors, NKEF-A and NKEF-B, members of the peroxiredoxin family. **Geiben-Lynn Ralf**; Kursar Mischo; Brown Nancy V; Addo Marylyn M; Shau Hungyi; Lieberman Judy; Luster Andrew D; Walker Bruce D. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, USA.. acceleration@rcn.com) . Journal of biological chemistry, (2003 Jan 17) 278 (3) 1569-74. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB CD8(+) T-cells are a major source for the production of non-cytolytic factors that inhibit HIV-1 replication. In order to characterize further these factors, we analyzed gene expression profiles of activated CD8(+) T-cells using a human cDNA expression array containing 588 human cDNAs. mRNA for the chemokine I-309 (CCL1), the cytokines granulocyte-macrophage colony-stimulating factor and interleukin-13, and natural killer cell enhancing factors (NKEF) -A and -B were up-regulated in bulk CD8(+) T-cells from HIV-1 seropositive individuals compared with seronegative individuals. Recombinant NKEF-A and NKEF-B inhibited HIV-1 replication when exogenously added to acutely infected T-cells at an ID(50) (dose inhibiting HIV-1 replication by 50%) of approximately 130 nm (3 microg/ml). Additionally, inhibition against dual-tropic simian immunodeficiency virus and dual-tropic simian-human immunodeficiency virus was found. T-cells transfected with NKEF-A or NKEF-B cDNA were able to inhibit 80-98% HIV-1 replication in vitro. Elevated plasma levels of both NKEF-A and NKEF-B proteins were detected in 23% of HIV-infected non-treated individuals but not in persons treated with highly active antiviral therapy or uninfected persons. These results indicate that the peroxiredoxin family members NKEF-A and NKEF-B are up-regulated in activated CD8(+) T-cells in HIV infection, and suggest that these antioxidant proteins contribute to the antiviral activity of CD8(+) T-cells.

L5 ANSWER 2 OF 5 MEDLINE on STN
2002677159. PubMed ID: 12437858. Anti-human immunodeficiency virus noncytolytic CD8+ T-cell response: a review. **Geiben-Lynn Ralf**. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.) AIDS patient care and STDs, (2002 Oct) 16 (10) 471-7. Ref: 61. Journal code: 9607225. ISSN: 1087-2914. Pub. country: United States. Language: English.
AB The CD8+ T-cell immune response for human immunodeficiency virus (HIV) is divided into a cytolytic and noncytolytic mechanism. The mechanism of cell-mediated cytotoxic immunity for the partial control of human immunodeficiency virus type 1 (HIV-1) replication in infected individuals

antigen-specific cytotoxic T-lymphocytes (CTL) is widely correlated with disease outcome. However, the mechanism of the noncytolytic component is not well understood. In part, this is because the main inhibitory factor or factors called CD8+ T-cell antiviral factor (CAF), have not yet been purified. In addition, results between the investigators are difficult to compare because of technical differences between laboratories, including the use of different in vitro cell expansion and stimulation methods for the CD8+ T cells, the necessity of sequential biochemical purification steps with restricted amounts of material, the complex analysis and interpretation of gene expression arrays, the use of different HIV strains, and the use of different short- or long-term inhibition assays using primary or immortalized target cells. Nevertheless, the diminishing efficacy of highly active antiretroviral therapy (HAART) because of the development of resistant HIV and the persistence of latent HIV provides a strong rationale for an immune therapy approach using antiviral factor(s) of the CD8+ T-cell noncytolytic immune response.

L5 ANSWER 3 OF 5 MEDLINE on STN

2002642657. PubMed ID: 12192009. Purification of a modified form of bovine antithrombin III as an HIV-1 CD8+ T-cell antiviral factor. **Geiben-Lynn Ralf**; Brown Nancy; Walker Bruce D; Luster Andrew D. (Partners AIDS Research Center, Center for Immunology and Inflammatory Diseases and Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital and Harvard Medical School, Boston 02129, USA.) Journal of biological chemistry, (2002 Nov 1) 277 (44) 42352-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB CD8(+) T-cells secrete soluble factor(s) capable of inhibiting both R5- and X4-tropic strains of human immunodeficiency virus type 1 (HIV-1). CCR5 chemokine ligands, released from activated CD8(+) T-cells, contribute to the antiviral activity of these cells. These CC-chemokines, however, do not account for all CD8(+) T-cell antiviral factor(s) (CAF) released from these cells, particularly because the elusive CAF can inhibit the replication of X4 HIV-1 strains that use CXCR4 and not CCR5 as a coreceptor. Here we demonstrate that activated CD8(+) T-cells of HIV-1-seropositive individuals modify serum bovine antithrombin III into an HIV-1 inhibitory factor capable of suppressing the replication of X4 HIV-1. These data indicate that antithrombin III may play a role in the progression of HIV-1 disease.

L5 ANSWER 5 OF 5 MEDLINE on STN

2001437909. PubMed ID: 11483776. Noncytolytic inhibition of X4 virus by bulk CD8(+) cells from human immunodeficiency virus type 1 (HIV-1)-infected persons and HIV-1-specific cytotoxic T lymphocytes is not mediated by beta-chemokines. **Geiben-Lynn R**; Kursar M; Brown N V; Kerr E L; Luster A D; Walker B D. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, USA.) Journal of virology, (2001 Sep) 75 (17) 8306-16. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Human immunodeficiency virus (HIV)-specific cytotoxic T lymphocytes (CTL) mediate immunologic selection pressure by both cytolytic and noncytolytic mechanisms. Non cytolytic mechanisms include the release of beta-chemokines blocking entry of R5 HIV-1 strains. In addition, CD8(+) cells inhibit X4 virus isolates via release of as yet poorly characterized soluble factors. To further characterize these factors, we performed detailed analysis of CTL as well as bulk CD8(+) T lymphocytes from six HIV-1-infected individuals and from six HIV-1-seronegative individuals. Kinetic studies revealed that secreted suppressive activities of HIV-1-specific CTL and bulk CD8(+) T lymphocytes from all HIV-1-infected persons are significantly higher than that of supernatants from seronegative controls. The suppressive activity could be blocked by monensin and brefeldin A, was heat labile, and appeared in a pattern different from that of secretion of chemokines (MDC, I-309, MIP-1alpha, MIP-1beta, and RANTES), cytokines (gamma interferon, tumor necrosis factor alpha, and granulocyte-macrophage colony-stimulating factor), and

activity was characterized by molecular size exclusion centrifugation and involves a suppressive activity of >50 kDa which could be bound to heparin and a nonbinding inhibitory activity of <50 kDa. Our data provide a functional link between CD8(+) cells and CTL in the noncytolytic inhibition of HIV-1 and suggest that suppression of X4 virus is mediated through proteins. The sizes of the proteins, their affinity for heparin, and the pattern of release indicate that these molecules are not chemokines.

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=> d his

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L2            7 S E3
L3            6 S L2 NOT L1

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                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4

=> e walker b d/au
E1            4     WALKER B C/AU
E2            1     WALKER B C JR/AU
E3          155 --> WALKER B D/AU
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E5          144     WALKER B F/AU
E6            3     WALKER B G/AU
E7            4     WALKER B H/AU
E8           14     WALKER B J/AU
E9           59     WALKER B JR/AU
E10          25     WALKER B K/AU
E11          68     WALKER B L/AU
E12           2     WALKER B LEE/AU

=> s e3
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=> s l6 not l5
L7          154 L6 NOT L5

=> s l7 and (peroxiredox? or NKEF? or natural killer enhancing factor?)
            336 PEROXIREDOX?
             19 NKEF?
         178186 NATURAL
          32159 KILLER
          34354 ENHANCING
        2122472 FACTOR?
             22 NATURAL KILLER ENHANCING FACTOR?
                  (NATURAL(W)KILLER(W)ENHANCING(W)FACTOR?)
L8            0 L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR
                ?)
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=> s l7 and (HIV or human immunodeficiency virus or SIV or simian immunodeficiency virus or len
        136286 HIV
       8502943 HUMAN
        112869 IMMUNODEFICIENCY
        373812 VIRUS
         42705 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
          3765 SIV
         18950 SIMIAN
        112869 IMMUNODEFICIENCY
        373812 VIRUS
          2322 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
          2881 LENTIVIR?
         32204 RETROVIR?
L9         127 L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
               IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR RETROVIR?)

=> s l9 and (CD8? or cytotoxic or antiviral? or suppressor? or soluble)
         41606 CD8?
         81762 CYTOTOXIC
         39479 ANTIVIRAL?
         52984 SUPPRESSOR?
        102720 SOLUBLE
L10        101 L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOLUBL
               E)

=> d l10,cbib,ab,1-101

L10  ANSWER 1 OF 101     MEDLINE on STN
2004101208.     PubMed ID: 14770175.   HIV evolution: CTL escape mutation and
     reversion after transmission. Leslie A J; Pfafferott K J; Chetty P;
     Draenert R; Addo M M; Feeney M; Tang Y; Holmes E C; Allen T; Prado J G;
     Altfeld M; Brander C; Dixon C; Ramduth D; Jeena P; Thomas S A; St John A;
     Roach T A; Kupfer B; Luzzi G; Edwards A; Taylor G; Lyall H; Tudor-Williams
     G; Novelli V; Martinez-Picado J; Kiepiela P; Walker B D; Goulder P J R.
     (Department of Pediatrics, Fuffield Department of Medicine, Peter Medawar
     Building for Pathogen Research, University of Oxford, Oxford OX1 3SY, UK.
     ) Nature medicine, (2004 Mar) 10 (3) 282-9. Journal code: 9502015. ISSN:
     1078-8956. Pub. country: United States. Language: English.
AB   Within-patient HIV evolution reflects the strong selection pressure
     driving viral escape from cytotoxic T-lymphocyte (CTL) recognition.
     Whether this intrapatient accumulation of escape mutations translates into
     HIV evolution at the population level has not been evaluated.  We
     studied over 300 patients drawn from the B- and C-clade epidemics,
     focusing on human leukocyte antigen (HLA) alleles HLA-B57 and HLA-B5801,
     which are associated with long-term HIV control and are therefore likely
     to exert strong selection pressure on the virus.  The CTL response
     dominating acute infection in HLA-B57/5801-positive subjects drove
     positive selection of an escape mutation that reverted to wild-type after
     transmission to HLA-B57/5801-negative individuals.  A second escape
     mutation within the epitope, by contrast, was maintained after
     transmission.  These data show that the process of accumulation of escape
     mutations within HIV is not inevitable.  Complex epitope- and
     residue-specific selection forces, including CTL-mediated positive
     selection pressure and virus-mediated purifying selection, operate in
     tandem to shape HIV evolution at the population level.

L10  ANSWER 2 OF 101     MEDLINE on STN
2004089531.     PubMed ID: 14963115.   Consistent cytotoxic-T-lymphocyte
     targeting of immunodominant regions in human immunodeficiency virus
     across multiple ethnicities. Frahm Nicole; Korber B T; Adams C M; Szinger
     J J; Draenert R; Addo M M; Feeney M E; Yusim K; Sango K; Brown N V;
     SenGupta D; Piechocka-Trocha A; Simonis T; Marincola F M; Wurcel A G;
     Stone D R; Russell C J; Adolf P; Cohen D; Roach T; StJohn A; Khatri A;
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Research Center, Endocrine Unit, Massachusetts General Hospital, Charlestown, Massachusetts 02129-2000, USA.) Journal of virology, (2004 Mar) 78 (5) 2187-200. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Although there is increasing evidence that virus-specific **cytotoxic**-T-lymphocyte (CTL) responses play an important role in the control of **human immunodeficiency virus** (**HIV**) replication in vivo, only scarce CTL data are available for the ethnic populations currently most affected by the epidemic. In this study, we examined the **CD8**(+)-T-cell responses in African-American, Caucasian, Hispanic, and Caribbean populations in which clade B virus dominates and analyzed the potential factors influencing immune recognition. Total **HIV**-specific **CD8**(+)-T-cell responses were determined by enzyme-linked immunospot assays in 150 **HIV**-infected individuals by using a clade B consensus sequence peptide set spanning all **HIV** proteins. A total of 88% of the 410 tested peptides were recognized, and Nef- and Gag-specific responses dominated the total response for each ethnicity in terms of both breadth and magnitude. Three dominantly targeted regions within these proteins that were recognized by >90% of individuals in each ethnicity were identified. Overall, the total breadth and magnitude of **CD8**(+)-T-cell responses correlated with individuals' CD4 counts but not with viral loads. The frequency of recognition for each peptide was highly correlated with the relative conservation of the peptide sequence, the presence of predicted immunoproteasomal cleavage sites within the C-terminal half of the peptide, and a reduced frequency of amino acids that impair binding of optimal epitopes to the restricting class I molecules. The present study thus identifies factors that contribute to the immunogenicity of these highly targeted and relatively conserved sequences in **HIV** that may represent promising vaccine candidates for ethnically heterogeneous populations.

L10 ANSWER 3 OF 101 MEDLINE on STN

2003610836. PubMed ID: 14694094. Persistent recognition of autologous virus by high-avidity **CD8** T cells in chronic, progressive **human immunodeficiency virus** type 1 infection. Draenert R; Verrill C L; Tang Y; Allen T M; Wurcel A G; Boczanowski M; Lechner A; Kim A Y; Suscovich T; Brown N V; Addo M M; **Walker B D**. (Howard Hughes Medical Institute, Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School Division of AIDS, Boston, Massachusetts 02129, USA.) Journal of virology, (2004 Jan) 78 (2) 630-41. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **CD8** T-cell responses are thought to be crucial for control of viremia in **human immunodeficiency virus** (**HIV**) infection but ultimately fail to control viremia in most infected persons. Studies in acute infection have demonstrated strong **CD8**-mediated selection pressure and evolution of mutations conferring escape from recognition, but the ability of **CD8** T-cell responses that persist in late-stage infection to recognize viruses present in vivo has not been determined. Therefore, we studied 24 subjects with advanced **HIV** disease (median viral load = 142,000 copies/ml; median CD4 count = 71/ micro l) and determined **HIV**-1-specific **CD8** T-cell responses to all expressed viral proteins using overlapping peptides by gamma interferon Elispot assay. Chronic-stage virus was sequenced to evaluate autologous sequences within Gag epitopes, and functional avidity of detected responses was determined. In these subjects, the median number of epitopic regions targeted was 13 (range, 2 to 39) and the median cumulative magnitude of **CD8** T-cell responses was 5,760 spot-forming cells/10(6) peripheral blood mononuclear cells (range, 185 to 24,700). On average six (range, one to 8) proteins were targeted. For 89% of evaluated **CD8** T-cell responses, the autologous viral sequence was predicted to be well recognized by these responses and the majority of analyzed optimal epitopes were recognized with medium to high functional avidity by the contemporary **CD8** T cells. Withdrawal of antigen by highly active antiretroviral therapy led to a significant decline both in breadth (P = 0.032) and magnitude (P = 0.0098) of these **CD8** T-cell responses, providing further evidence that these responses had been driven

by recognition of autologous virus. These results indicate that strong, broadly directed, and high-avidity gamma-interferon-positive **CD8** T-cells directed at autologous virus persist in late disease stages, and the absence of mutations within viral epitopes indicates a lack of strong selection pressure mediated by these responses. These data imply functional impairment of **CD8** T-cell responses in late-stage infection that may not be reflected by gamma interferon-based screening techniques.

L10 ANSWER 4 OF 101 MEDLINE on STN

2003278178. PubMed ID: 12805449. Comprehensive screening reveals strong and broadly directed **human immunodeficiency virus** type 1-specific **CD8** responses in perinatally infected children. Feeney M E; Roosevelt K A; Tang Y; Pfafferott K J; McIntosh K; Burchett S K; Mao C; **Walker B D**; Goulder P J R. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical Schoo, Boston, Massachusetts, USA.) Journal of virology, (2003 Jul) 77 (13) 7492-501. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Advances in **antiviral** therapy have dramatically shifted the demographics of pediatric **human immunodeficiency virus** type 1 (**HIV**-1) infection in the developed world, and a growing proportion of perinatally **HIV**-1-infected children are now entering their second or even third decade of life. Although cellular immune responses to **HIV** are known to be weak in early infancy, the magnitude, breadth, and specificity of responses later in childhood have not been characterized in detail. We performed a comprehensive characterization of **HIV**-1-specific **CD8** responses in 18 perinatally infected children (age range, 6 to 17 years), most of whom were on **antiviral** therapy, using both previously defined **HIV**-1 epitopes and overlapping peptides spanning all **HIV**-1 proteins. Multispecific responses were detected in all subjects and accounted for a median of 0.25 to 0.3% of all peripheral blood mononuclear cells that was similar to the magnitude seen in **HIV**-infected adults. **CD8** responses were broadly directed at an average of 11 epitopes (range, 2 to 27 epitopes) and targeted nearly all **HIV**-1 proteins, with the highest proportion in Gag. Responses were readily detected even in those children with suppressed viremia on highly active antiretroviral therapy, although the breadth ($P = 0.037$) and the magnitude ($P = 0.021$) were significantly lower in these subjects. Each child recognized only a small minority of the **HIV**-1 optimal epitopes defined for his or her class I HLA alleles. Together, these data indicate that perinatally infected children who survive infancy mount a robust **HIV**-1-specific **CD8** response that is much stronger than previously thought and is comparable in magnitude and breadth to that of adults. Moreover, this response has the potential to be broadened to target more epitopes, making these children attractive candidates for immunotherapeutic interventions.

L10 ANSWER 5 OF 101 MEDLINE on STN

2003019244. PubMed ID: 12525643. Comprehensive epitope analysis of **human immunodeficiency virus** type 1 (**HIV**-1)-specific T-cell responses directed against the entire expressed **HIV**-1 genome demonstrate broadly directed responses, but no correlation to viral load. Addo M M; Yu X G; Rathod A; Cohen D; Eldridge R L; Strick D; Johnston M N; Corcoran C; Wurcel A G; Fitzpatrick C A; Feeney M E; Rodriguez W R; Basgoz N; Draenert R; Stone David R; Brander C; Goulder P J R; Rosenberg E S; Altfeld M; **Walker B D**. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School. Fenway Community Health Center. Lemuel Shattuck Hospital, Boston, Massachusetts 02129, USA.) Journal of virology, (2003 Feb) 77 (3) 2081-92. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Cellular immune responses play a critical role in the control of **human immunodeficiency virus** type 1 (**HIV**-1); however, the breadth of these responses at the single-epitope level has not been comprehensively assessed. We therefore screened peripheral blood mononuclear cells (PBMC) from 57 individuals at different stages of **HIV**-1 infection for virus-specific T-cell responses using a matrix of 504 overlapping peptides spanning all expressed **HIV**-1 proteins in a gamma interferon-enzyme-

linked immunospot (ELISpot) assay. **HIV**-1 specific T-cell responses were detectable in all study subjects, with a median of 14 individual epitopic regions targeted per person (range, 2 to 42), and all 14 **HIV**-1 protein subunits were recognized. **HIV**-1 p24-Gag and Nef contained the highest epitope density and were also the most frequently recognized **HIV**-1 proteins. The total magnitude of the **HIV**-1-specific response ranged from 280 to 25,860 spot-forming cells (SFC)/10(6) PBMC (median, 4,245) among all study participants. However, the number of epitopic regions targeted, the protein subunits recognized, and the total magnitude of **HIV**-1-specific responses varied significantly among the tested individuals, with the strongest and broadest responses detectable in individuals with untreated chronic **HIV**-1 infection. Neither the breadth nor the magnitude of the total **HIV**-1-specific **CD8**+-T-cell responses correlated with plasma viral load. We conclude that a peptide matrix-based Elispot assay allows for rapid, sensitive, specific, and efficient assessment of cellular immune responses directed against the entire expressed **HIV**-1 genome. These data also suggest that the impact of T-cell responses on control of viral replication cannot be explained by the mere quantification of the magnitude and breadth of the **CD8**+-T-cell response, even if a comprehensive pan-genome screening approach is applied.

L10 ANSWER 6 OF 101 MEDLINE on STN

2002637571. PubMed ID: 12396610. **Cytotoxic** T-lymphocyte (CTL) responses directed against regulatory and accessory proteins in **HIV**-1 infection. Addo M M; Yu X G; Rosenberg E S; **Walker B D**; Altfeld M. (Partners AIDS Research Center, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, USA.. addo@helix.mgh.harvard.edu) . DNA and cell biology, (2002 Sep) 21 (9) 671-8. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB The **HIV**-1 regulatory proteins Tat and Rev and the accessory proteins Vpr, Vpu, and Vif are essential for viral replication, and their cytoplasmic production suggests that they should be processed for recognition by **cytotoxic** T lymphocytes. However, only limited data is available evaluating to which extent these proteins are targeted in natural infection and optimal **cytotoxic** T lymphocyte (CTL) epitopes within these proteins have not been defined. In this study, CTL responses against **HIV**-1 Tat, Rev, Vpr, Vpu, and Vif were analyzed in 70 **HIV**-1 infected individuals and 10 **HIV**-1 negative controls using overlapping peptides spanning the entire proteins. Peptide-specific interferon-gamma (IFN-gamma) production was measured by Elispot assay and flow-based intracellular cytokine quantification. HLA class I restriction and **cytotoxic** activity were confirmed after isolation of peptide-specific **CD8**+ T-cell lines. All regulatory and accessory proteins served as targets for **HIV**-1- specific CTL and multiple CTL epitopes were identified in functionally important regions of these proteins. In certain individuals **HIV**-1-specific **CD8**+ T-cell responses to these accessory and regulatory proteins contributed up to a third to the magnitude of the total **HIV**-1-specific CTL response. These data indicate that despite the small size of these proteins regulatory and accessory proteins are targeted by CTL in natural **HIV**-1 infection, and contribute importantly to the total **HIV**-1-specific **CD8**+ T-cell responses. These findings are relevant for the evaluation of the specificity and breadth of immune responses during acute and chronic#10; infection, and will be useful for the design and testing of candidate **human immunodeficiency virus** (**HIV**) vaccines.

L10 ANSWER 7 OF 101 MEDLINE on STN

2001548908. PubMed ID: 11595297. Mother-to-child transmission of **HIV** infection and CTL escape through HLA-A2-SLYNTVATL epitope sequence variation. Goulder P J; Pasquier C; Holmes E C; Liang B; Tang Y; Izopet J; Saune K; Rosenberg E S; Burchett S K; McIntosh K; Barnardo M; Bunce M; **Walker B D**; Brander C; Phillips R E. (Department of Paediatrics, Nuffield Department of Medicine, Level 7, Room 7615, John Radcliffe Hospital, Oxford OX3 9DU, UK.. philip.goulder@ndm.ox.ac.uk) . Immunology letters, (2001 Nov 1) 79 (1-2) 109-16. Journal code: 7910006. ISSN:

Pub. country: Netherlands. Language: English.

AB **Cytotoxic** T lymphocytes (CTL) play a central role in containment of **HIV** infection. Evasion of the immune response by CTL escape is associated with progression to disease. It is therefore hypothesised that transmitted viruses encode escape mutations within epitopes that are required for successful control of viraemia. In order to test this hypothesis, escape through the dominant HLA-A2-restricted CTL epitope SLYNTVATL (p17 Gag residues 77-85 SL9) in the setting of mother-to-child-transmission (MTCT) was investigated. Initial data from two families in which the **HIV**-infected mother expressed HLA-A*0201 and had transmitted the virus to other family members were consistent with this hypothesis. In addition, analysis of the gag sequence phylogeny in one family demonstrated that CTL escape variants can be successfully transmitted both horizontally and vertically. To test the hypothesis further, a larger cohort of transmitting mothers (n=8) and non-transmitters (n=14) were studied. Variation within the SL9 epitope was associated with expression of HLA-A2 (P=0.04) but overall no clear link between variation from the SL9 consensus sequence and MTCT was established. However, the high level of background diversity within p17 Gag served to obscure any possible association between escape and MTCT. In conclusion, these studies highlighted the obstacles to demonstrating CTL escape arising at this particular epitope. Alternative strategies likely to be more definitive are discussed.

L10 ANSWER 8 OF 101 MEDLINE on STN

2001534412. PubMed ID: 11581388. Neutralizing antibodies associated with viremia control in a subset of individuals after treatment of acute **human immunodeficiency virus** type 1 infection. Montefiori D C; Hill T S; Vo H T; **Walker B D**; Rosenberg E S. (Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, USA.. monte@duke.edu) . Journal of virology, (2001 Nov) 75 (21) 10200-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Immediate treatment of acute **human immunodeficiency virus** type 1 (**HIV**-1) infection has been associated with subsequent control of viremia in a subset of patients after therapy cessation, but the immune responses contributing to control have not been fully defined. Here we examined neutralizing antibodies as a correlate of viremia control following treatment interruption in **HIV**-1-infected individuals in whom highly active antiretriviral therapy (HAART) was initiated during early seroconversion and who remained on therapy for 1 to 3 years. Immediately following treatment interruption, neutralizing antibodies were undetectable with T-cell-line adapted strains and the autologous primary **HIV**-1 isolate in seven of nine subjects. Env- and Gag-specific antibodies as measured by enzyme-linked immunosorbent assay were also low or undetectable at this time. Despite this apparent poor maturation of the virus-specific B-cell response during HAART, autologous neutralizing antibodies emerged rapidly and correlated with a spontaneous downregulation in rebound viremia following treatment interruption in three subjects. Control of rebound viremia was seen in other subjects in the absence of detectable neutralizing antibodies. The results indicate that virus-specific B-cell priming occurs despite the early institution of HAART, allowing rapid secondary neutralizing-antibody production following treatment interruption in a subset of individuals. Since early HAART limits viral diversification, we hypothesize that potent neutralizing-antibody responses to autologous virus are able to mature and that in some persons these responses contribute to the control of plasma viremia after treatment cessation.

L10 ANSWER 9 OF 101 MEDLINE on STN

2001512177. PubMed ID: 11559810. Multiple effector functions mediated by **human immunodeficiency virus**-specific CD4(+) T-cell clones. Norris P J; Sumaroka M; Brander C; Moffett H F; Boswell S L; Nguyen T; Sykulev Y; **Walker B D**; Rosenberg E S. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA.) Journal of virology, (2001 Oct) 75

States. Language: English.

AB Mounting evidence suggests that **human immunodeficiency virus** type 1 (**HIV**-1) Gag-specific T helper cells contribute to effective **antiviral** control, but their functional characteristics and the precise epitopes targeted by this response remain to be defined. In this study, we generated CD4(+) T-cell clones specific for Gag from **HIV**-1-infected persons with vigorous Gag-specific responses detectable in peripheral blood mononuclear cells. Multiple peptides containing T helper epitopes were identified, including a minimal peptide, VHAGPIAG (amino acids 218 to 226), in the cyclophilin binding domain of Gag. Peptide recognition by all clones examined induced cell proliferation, gamma interferon (IFN-gamma) secretion, and cytolytic activity. Cytolysis was abrogated by concanamycin A and EGTA but not brefeldin A or anti-Fas antibody, implying a perforin-mediated mechanism of cell lysis. Additionally, serine esterase release into the extracellular medium, a marker for cytolytic granules, was demonstrated in an antigen-specific, dose-dependent fashion. These data indicate that T helper cells can target multiple regions of the p24 Gag protein and suggest that cytolytic activity may be a component of the **antiviral** effect of these cells.

L10 ANSWER 10 OF 101 MEDLINE on STN

2001481742. PubMed ID: 11509618. Vpr is preferentially targeted by CTL during **HIV**-1 infection. Altfeld M; Addo M M; Eldridge R L; Yu X G; Thomas S; Khatri A; Strick D; Phillips M N; Cohen G B; Islam S A; Kalams S A; Brander C; Goulder P J; Rosenberg E S; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129, USA. (HIV Study Collaboration).) Journal of immunology (Baltimore, Md. : 1950), (2001 Sep 1) 167 (5) 2743-52. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The **HIV**-1 accessory proteins Vpr, Vpu, and Vif are essential for viral replication, and their cytoplasmic production suggests that they should be processed for recognition by CTLs. However, the extent to which these proteins are targeted in natural infection, as well as precise CTL epitopes within them, remains to be defined. In this study, CTL responses against **HIV**-1 Vpr, Vpu, and Vif were analyzed in 60 **HIV**-1-infected individuals and 10 **HIV**-1-negative controls using overlapping peptides spanning the entire proteins. Peptide-specific IFN-gamma production was measured by ELISPOT assay and flow-based intracellular cytokine quantification. HLA class I restriction and **cytotoxic** activity were confirmed after isolation of peptide-specific **CD8**(+) T cell lines. **CD8**(+) T cell responses against Vpr, Vpu, and Vif were found in 45%, 2%, and 33% of **HIV**-1-infected individuals, respectively. Multiple CTL epitopes were identified in functionally important regions of **HIV**-1 Vpr and Vif. Moreover, in infected individuals in whom the breadth of **HIV**-1-specific responses was assessed comprehensively, Vpr and p17 were the most preferentially targeted proteins per unit length by **CD8**(+) T cells. These data indicate that despite the small size of these proteins Vif and Vpr are frequently targeted by CTL in natural **HIV**-1 infection and contribute importantly to the total **HIV**-1-specific **CD8**(+) T cell responses. These findings will be important in evaluating the specificity and breadth of immune responses during acute and chronic infection, and in the design and testing of candidate **HIV** vaccines.

L10 ANSWER 11 OF 101 MEDLINE on STN

2001431994. PubMed ID: 11479610. Less is more? STI in acute and chronic **HIV**-1 infection. Altfeld M; **Walker B D**. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.. maltfeld@partners.org) . Nature medicine, (2001 Aug) 7 (8) 881-4. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

L10 ANSWER 12 OF 101 MEDLINE on STN

2001424537. PubMed ID: 11460164. Evolution and transmission of stable CTL escape mutations in **HIV** infection. Goulder P J; Brander C; Tang Y;

Trocha A; Altfeld M; He S; Bunce M; Funkhouser R; Pelton S I; Burchett S K; McIntosh K; Korber B T; **Walker B D.** (Partners AIDS Research Center, Massachusetts General Hospital and Division of AIDS, Harvard Medical School, Boston, Massachusetts 02114, USA.. goulder@helix.mg.harvard.edu) . Nature, (2001 Jul 19) 412 (6844) 334-8. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB Increasing evidence indicates that potent anti-**HIV**-1 activity is mediated by **cytotoxic** T lymphocytes (CTLs); however, the effects of this immune pressure on viral transmission and evolution have not been determined. Here we investigate mother-child transmission in the setting of human leukocyte antigen (HLA)-B27 expression, selected for analysis because it is associated with prolonged immune containment in adult infection. In adults, mutations in a dominant and highly conserved B27-restricted Gag CTL epitope lead to loss of recognition and disease progression. In mothers expressing HLA-B27 who transmit **HIV**-1 perinatally, we document transmission of viruses encoding CTL escape variants in this dominant Gag epitope that no longer bind to B27. Their infected infants target an otherwise subdominant B27-restricted epitope and fail to contain **HIV** replication. These CTL escape variants remain stable without reversion in the absence of the evolutionary pressure that originally selected the mutation. These data suggest that CTL escape mutations in epitopes associated with suppression of viraemia will accumulate as the epidemic progresses, and therefore have important implications for vaccine design.

L10 ANSWER 13 OF 101 MEDLINE on STN

2001385588. PubMed ID: 11439948. Hepatitis C virus infection. Lauer G M; **Walker B D.** (Infectious Disease Division and Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, USA.) New England journal of medicine, (2001 Jul 5) 345 (1) 41-52. Ref: 106. Journal code: 0255562. ISSN: 0028-4793. Pub. country: United States. Language: English.

L10 ANSWER 14 OF 101 MEDLINE on STN

2001349953. PubMed ID: 11413294. Relative dominance of epitope-specific **cytotoxic** T-lymphocyte responses in **human immunodeficiency virus** type 1-infected persons with shared HLA alleles. Day C L; Shea A K; Altfeld M A; Olson D P; Buchbinder S P; Hecht F M; Rosenberg E S; **Walker B D**; Kalams S A. (Partners AIDS Research Center, Massachusetts General Hospital, Boston, MA 02114, USA.) Journal of virology, (2001 Jul) 75 (14) 6279-91. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Cytotoxic** T lymphocytes (CTL) target multiple epitopes in **human immunodeficiency virus** (**HIV**)-infected persons, and are thought to influence the viral set point. The extent to which HLA class I allele expression predicts the epitopes targeted has not been determined, nor have the relative contributions of responses restricted by different class I alleles within a given individual. In this study, we performed a detailed analysis of the CTL response to optimally defined CTL epitopes restricted by HLA class I A and B alleles in individuals who coexpressed HLA A2, A3, and B7. The eight **HIV**-1-infected subjects studied included two subjects with acute **HIV** infection, five subjects with chronic **HIV** infection, and one long-term nonprogressor. Responses were heterogeneous with respect to breadth and magnitude of CTL responses in individuals of the same HLA type. Of the 27 tested epitopes that are presented by A2, A3, and B7, 25 were targeted by at least one person. However, there was wide variation in the number of epitopes targeted, ranging from 2 to 17. The A2-restricted CTL response, which has been most extensively studied in infected persons, was found to be narrowly directed in most individuals, and in no cases was it the dominant contributor to the total **HIV**-1-specific CTL response. These results indicate that HLA type alone does not predict CTL responses and that numerous potential epitopes may not be targeted by CTL in a given individual. These data also provide a rationale for boosting both the breadth and the magnitude of **HIV**-1-specific CTL responses by immunotherapy in persons with chronic

L10 ANSWER 15 OF 101 MEDLINE on STN

2001276055. PubMed ID: 11360218. **Human immunodeficiency virus** pathogenesis and prospects for immune control in patients with established infection. Cohen D E; **Walker B D**. (Fenway Community Health Center, Boston, MA 02114, USA.. dcohen@fenwayhealth.org) . Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, (2001 Jun 15) 32 (12) 1756-68. Ref: 126. Journal code: 9203213. ISSN: 1058-4838. Pub. country: United States. Language: English.

AB Infection with **human immunodeficiency virus** (**HIV**) results in inevitable progressive deterioration of the immune system in the majority of untreated patients. Prospects for virus eradication are remote, because **HIV** establishes long-lived reservoirs during the earliest stages of infection that are impervious to available **antiviral** therapies. Understanding how the immune system copes with this illness and other chronic viral infections is the key to designing future strategies for long-term control of viremia. Valuable insights have been gained from 2 populations in particular: patients with chronic, long-term, nonprogressing infections, in whom viremia is controllable in the absence of **antiviral** medications, and acutely infected patients, in whom the initial **HIV**-specific immune response might be preserved and augmented by timely intervention. These cases of immune control of **HIV** provide hope for the development of improved vaccine products that may eventually produce vaccine-induced immunity that will enhance durable control of **HIV** infection.

L10 ANSWER 16 OF 101 MEDLINE on STN

2001256877. PubMed ID: 11148222. Substantial differences in specificity of **HIV**-specific **cytotoxic** T cells in acute and chronic **HIV** infection. Goulder P J; Altfeld M A; Rosenberg E S; Nguyen T; Tang Y; Eldridge R L; Addo M M; He S; Mukherjee J S; Phillips M N; Bunce M; Kalams S A; Sekaly R P; **Walker B D**; Brander C. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, USA.. goulder@helix.mgh.harvard.edu) . Journal of experimental medicine, (2001 Jan 15) 193 (2) 181-94. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB **Cytotoxic** T lymphocytes (CTLs) play a vital part in controlling viral replication during human viral infections. Most studies in human infections have focused on CTL specificities in chronic infection and few data exist regarding the specificity of the initial CTL response induced in acute infection. In this study, **HIV**-1 infection in persons expressing human histocompatibility leukocyte antigen (HLA)-A*0201 was used as a means of addressing this issue. In chronic infection, the dominant HLA-A*0201-restricted CTL response is directed towards the epitope SLYNTVATL ("SL9") in p17 Gag (residues 77-85). This epitope is targeted by 75% of HLA-A*0201-positive adults, and the magnitude of this A*0201-SL9 response shows a strong negative association with viral load in progressive infection. Despite using the highly sensitive peptide-major histocompatibility complex tetramer and intracellular cytokine assays, responses to the SL9 epitope were not detectable in any of 11 HLA-A*0201-positive subjects with acute **HIV**-1 infection ($P = 2 \times 10(-6)$), even when assays were repeated using the SL9 peptide variant that was encoded by their autologous virus. In contrast, multiple responses (median 3) to other epitopes were evident in 7 of the 11 A*0201-positive subjects. Longitudinal study of two subjects confirmed that the A*0201-SL9 response emerged later than other CTL responses, and after viral set point had been reached. Together, these data show that the CTL responses that are present and that even may dominate in chronic infection may differ substantially from those that constitute the initial **antiviral** CTL response. This finding is an important consideration in vaccine design and in the evaluation of vaccine candidates.

L10 ANSWER 17 OF 101 MEDLINE on STN

2001256876. PubMed ID: 11148221. Cellular immune responses and viral diversity in individuals treated during acute and early **HIV**-1 infection.

Eldridge R L; Addo M M; Poon S H; Phillips M N; Robbins G K; Sax P E; Boswell S; Kahn J O; Brander C; Goulder P J; Levy J A; Mullins J I; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, USA.) Journal of experimental medicine, (2001 Jan 15) 193 (2) 169-80. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Immune responses induced during the early stages of chronic viral infections are thought to influence disease outcome. Using **HIV** as a model, we examined virus-specific **cytotoxic** T lymphocytes (CTLs), T helper cells, and viral genetic diversity in relation to duration of infection and subsequent response to **antiviral** therapy. Individuals with acute **HIV**-1 infection treated before seroconversion had weaker CTL responses directed at fewer epitopes than persons who were treated after seroconversion. However, treatment-induced control of viremia was associated with the development of strong T helper cell responses in both groups. After 1 yr of **antiviral** treatment initiated in acute or early infection, all epitope-specific CTL responses persisted despite undetectable viral loads. The breadth and magnitude of CTL responses remained significantly less in treated acute infection than in treated chronic infection, but viral diversity was also significantly less with immediate therapy. We conclude that early treatment of acute **HIV** infection leads to a more narrowly directed CTL response, stronger T helper cell responses, and a less diverse virus population. Given the need for T helper cells to maintain effective CTL responses and the ability of virus diversification to accommodate immune escape, we hypothesize that early therapy of primary infection may be beneficial despite induction of less robust CTL responses. These data also provide rationale for therapeutic immunization aimed at broadening CTL responses in treated primary **HIV** infection.

L10 ANSWER 18 OF 101 MEDLINE on STN

2001214511. PubMed ID: 11172028. The **HIV**-1 regulatory proteins Tat and Rev are frequently targeted by **cytotoxic** T lymphocytes derived from **HIV**-1-infected individuals. Addo M M; Altfeld M; Rosenberg E S; Eldridge R L; Philips M N; Habeeb K; Khatri A; Brander C; Robbins G K; Mazzara G P; Goulder P J; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA. (HIV Controller Study Collaboration).) Proceedings of the National Academy of Sciences of the United States of America, (2001 Feb 13) 98 (4) 1781-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The **HIV**-1 regulatory proteins Rev and Tat are expressed early in the virus life cycle and thus may be important targets for the immune control of **HIV**-1-infection and for effective vaccines. However, the extent to which these proteins are targeted in natural **HIV**-1 infection as well as precise epitopes targeted by human **cytotoxic** T lymphocytes (CTL) remain to be defined. In the present study, 57 **HIV**-1-infected individuals were screened for responses against Tat and Rev by using overlapping peptides spanning the entire Tat and Rev proteins. **CD8**+ T cell responses against Tat and Rev were found in up to 19 and 37% of **HIV**-1-infected individuals, respectively, indicating that these regulatory proteins are important targets for **HIV**-1-specific CTL. Despite the small size of these proteins, multiple CTL epitopes were identified in each. These data indicate that Tat and Rev are frequently targeted by CTL in natural **HIV**-1 infection and may be important targets for **HIV** vaccines.

L10 ANSWER 19 OF 101 MEDLINE on STN

2001142661. PubMed ID: 11160150. **HIV** versus the immune system: another apparent victory for the virus. Letvin N L; **Walker B D**. (Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA.) Journal of clinical investigation, (2001 Feb) 107 (3) 273-5. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

L10 ANSWER 20 OF 101 MEDLINE on STN
2001111651. PubMed ID: 11152507. Rapid definition of five novel HLA-A*3002-restricted **human immunodeficiency virus**-specific **cytotoxic** T-lymphocyte epitopes by elispot and intracellular cytokine staining assays. Goulder P J; Addo M M; Altfeld M A; Rosenberg E S; Tang Y; Govender U; Mngqundaniso N; Annamalai K; Vogel T U; Hammond M; Bunce M; Coovadia H M; **Walker B D**. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, USA.. philip.goulder@ndm.ox.ac.uk) . Journal of virology, (2001 Feb) 75 (3) 1339-47. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** (**HIV**)-specific **cytotoxic** T lymphocytes (CTL) play a major role in control of viral replication. To understand the contribution of this **antiviral** response, an initial step is to fully define the specific epitopes targeted by CTL. These studies focused on CTL responses restricted by HLA-A*3002, one of the HLA-A molecules most prominent in African populations. To avoid the time-consuming effort and expense involved in culturing CTL prior to defining epitopes and restricting alleles, we developed a method combining Elispot assays with intracellular gamma interferon staining of peripheral blood mononuclear cells to first map the optimal epitopes targeted and then define the HLA restriction of novel epitopes. In two A*3002-positive subjects whose CTL responses were characterized in detail, the strongest response in both cases was to an epitope in p17 Gag, RSLYNTVATLY (residues 76 to 86). Using this method, CTL epitopes for which there were no motif predictions were optimized and the HLA restriction was established within 48 to 72 h of receipt of blood. This simple and convenient approach should prove useful especially in the characterization of CTL responses specific to **HIV** and other viruses, particularly in localities where performing cytotoxicity assays would be problematic.

L10 ANSWER 21 OF 101 MEDLINE on STN
2001111647. PubMed ID: 11152503. Identification of novel HLA-A2-restricted **human immunodeficiency virus** type 1-specific **cytotoxic** T-lymphocyte epitopes predicted by the HLA-A2 supertype peptide-binding motif. Altfeld M A; Livingston B; Reshamwala N; Nguyen P T; Addo M M; Shea A; Newman M; Fikes J; Sidney J; Wentworth P; Chesnut R; Eldridge R L; Rosenberg E S; Robbins G K; Brander C; Sax P E; Boswell S; Flynn T; Buchbinder S; Goulder P J; **Walker B D**; Sette A; Kalams S A. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.) Journal of virology, (2001 Feb) 75 (3) 1301-11. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Virus-specific **cytotoxic** T-lymphocyte (CTL) responses are critical in the control of **human immunodeficiency virus** type 1 (**HIV**-1) infection and will play an important part in therapeutic and prophylactic **HIV**-1 vaccines. The identification of virus-specific epitopes that are efficiently recognized by CTL is the first step in the development of future vaccines. Here we describe the immunological characterization of a number of novel **HIV**-1-specific, HLA-A2-restricted CTL epitopes that share a high degree of conservation within **HIV**-1 and a strong binding to different alleles of the HLA-A2 superfamily. These novel epitopes include the first reported CTL epitope in the Vpr protein. Two of the novel epitopes were immunodominant among the HLA-A2-restricted CTL responses of individuals with acute and chronic **HIV**-1 infection. The novel CTL epitopes identified here should be included in future vaccines designed to induce **HIV**-1-specific CTL responses restricted by the HLA-A2 superfamily and will be important to assess in immunogenicity studies in infected persons and in uninfected recipients of candidate **HIV**-1 vaccines.

L10 ANSWER 22 OF 101 MEDLINE on STN
2001089127. PubMed ID: 11120778. Functionally inert **HIV**-specific **cytotoxic** T lymphocytes do not play a major role in chronically infected adults and children. Goulder P J; Tang Y; Brander C; Betts M R; Altfeld M; Annamalai K; Trocha A; He S; Rosenberg E S; Ogg G; O'Callaghan C A; Kalams S A; McKinney R E Jr; Mayer K; Koup R A; Pelton S I; Burchett S K;

Robinson H; **Walker B D**. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, USA.. goulder@helix.mgh.harvard.edu) . Journal of experimental medicine, (2000 Dec 18) 192 (12) 1819-32. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB The highly sensitive quantitation of virus-specific **CD8**(+) T cells using major histocompatibility complex-peptide tetramer assays has revealed higher levels of **cytotoxic** T lymphocytes (CTLs) in acute and chronic virus infections than were recognized previously. However, studies in lymphocytic choriomeningitis virus infection have shown that tetramer assays may include measurement of a substantial number of tetramer-binding cells that are functionally inert. Such phenotypically silent CTLs, which lack cytolytic function and do not produce interferon (IFN)-gamma, have been hypothesized to explain the persistence of virus in the face of a quantitatively large immune response, particularly when CD4 help is impaired. In this study, we examined the role of functionally inert CTLs in chronic **HIV** infection. Subjects studied included children and adults (n = 42) whose viral loads ranged from <50 to >100,000 RNA copies/ml plasma. Tetramer assays were compared with three functional assays: enzyme-linked immunospot (Elispot), intracellular cytokine staining, and precursor frequency (limiting dilution assay [LDA]) cytotoxicity assays. Strong positive associations were observed between cell numbers derived by the Elispot and the tetramer assay (r = 0.90). An even stronger association between tetramer-derived numbers and intracellular cytokine staining for IFN-gamma was present (r = 0.97). The majority (median 76%) of tetramer-binding cells were consistently detectable via intracellular IFN-gamma cytokine staining. Furthermore, modifications to the LDA, using a low input cell number into each well, enabled LDAs to reach equivalence with the other methods of CTL enumeration. These data together show that functionally inert CTLs do not play a significant role in chronic pediatric or adult **HIV** infection.

L10 ANSWER 23 OF 101 MEDLINE on STN

2001076806. PubMed ID: 11086046. The role of CD4+ T cell help and CD40 ligand in the in vitro expansion of **HIV**-1-specific memory **cytotoxic CD8**+ T cell responses. Ostrowski M A; Justement S J; Ehler L; Mizell S B; Lui S; Mican J; **Walker B D**; Thomas E K; Seder R; Fauci A S. (Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.. m.ostrowski@utoronto.ca) . Journal of immunology (Baltimore, Md. : 1950), (2000 Dec 1) 165 (11) 6133-41. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB CD4(+) T cells have been shown to play a critical role in the maintenance of an effective anti-viral **CD8**(+) CTL response in murine models. Recent studies have demonstrated that CD4(+) T cells provide help to CTLs through ligation of the CD40 receptor on dendritic cells. The role of CD4(+) T cell help in the expansion of virus-specific **CD8**(+) memory T cell responses was examined in normal volunteers recently vaccinated to influenza and in **HIV**-1 infected individuals. In recently vaccinated normal volunteers, CD4(+) T cell help was required for optimal in vitro expansion of influenza-specific CTL responses. Also, CD40 ligand trimer (CD40LT) enhanced CTL responses and was able to completely substitute for CD4(+) T cell help in PBMCs from normal volunteers. In **HIV**-1 infection, CD4(+) T cell help was required for optimal expansion of **HIV**-1-specific memory CTL in vitro in 9 of 10 patients. CD40LT could enhance CTL in the absence of CD4(+) T cell help in the majority of patients; however, the degree of enhancement of CTL responses was variable such that, in some patients, CD40LT could not completely substitute for CD4(+) T cell help. In those **HIV**-1-infected patients who demonstrated poor responses to CD40LT, a dysfunction in circulating **CD8**(+) memory T cells was demonstrated, which was reversed by the addition of cytokines including IL-2. Finally, it was demonstrated that IL-15 produced by CD40LT-stimulated dendritic cells may be an additional mechanism by which CD40LT induces the expansion of memory CTL in CD4(+) T cell-depleted conditions, where IL-2 is lacking.

2001046339. PubMed ID: 11023458. Cellular immunity to **human immunodeficiency virus** type 1 (**HIV**-1) clades: relevance to **HIV**-1 vaccine trials in Uganda. Cao H; Mani I; Vincent R; Mugerwa R; Mugyenyi P; Kanki P; Ellner J; **Walker B D**. (Massachusetts General Hospital-East, AIDS Research Center, Charlestown, MA 02129, USA.. cao@helix.mgh.harvard.edu) . Journal of infectious diseases, (2000 Nov) 182 (5) 1350-6. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB The first prophylactic **human immunodeficiency virus** type 1 (**HIV**-1) vaccine trial in Africa, with a clade B immunogen, is currently under way in Uganda, in a region where clades A and D are endemic. The use of a B clade vaccine is based on anticipated cross-recognition of endemic strains of **HIV**-1 in Uganda, but, in fact, little is known about the **cytotoxic** T lymphocyte (CTL) responses in that region. Seventeen **HIV**-1-infected volunteers from Kampala, Uganda, were studied to determine the immune responses elicited by natural infection with local **HIV**-1 strains. Despite the presence of broad cross-clade recognition, the CTL responses to the infecting viral clade were highest in most people. Recognition of nonendemic clade B antigens was similar to that of the coendemic local clade, and, in some instances, cross-recognition of clade B was greater. Nevertheless, the degree of cross-clade cellular responses we observed lends justification to the use of clade B-based immunogens in the current phase 1 vaccine trial in Uganda.

L10 ANSWER 25 OF 101 MEDLINE on STN

2000477635. PubMed ID: 11029005. Immune control of **HIV**-1 after early treatment of acute infection. Rosenberg E S; Altfeld M; Poon S H; Phillips M N; Wilkes B M; Eldridge R L; Robbins G K; D'Aquila R T; Goulder P J; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Boston 02114, USA.) Nature, (2000 Sep 28) 407 (6803) 523-6. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Virus-specific T-helper cells are considered critical for the control of chronic viral infections. Successful treatment of acute **HIV**-1 infection leads to augmentation of these responses, but whether this enhances immune control has not been determined. We administered one or two supervised treatment interruptions to eight subjects with treated acute infection, with the plan to restart therapy if viral load exceeded 5,000 copies of **HIV**-1 RNA per millilitre of plasma (the level at which therapy has been typically recommended) for three consecutive weeks, or 50,000 RNA copies per ml at one time. Here we show that, despite rebound in viraemia, all subjects were able to achieve at least a transient steady state off therapy with viral load below 5,000 RNA copies per ml. At present, five out of eight subjects remain off therapy with viral loads of less than 500 RNA copies per ml plasma after a median 6.5 months (range 5-8.7 months). We observed increased virus-specific **cytotoxic** T lymphocytes and maintained T-helper-cell responses in all. Our data indicate that functional immune responses can be augmented in a chronic viral infection, and provide rationale for immunotherapy in **HIV**-1 infection.

L10 ANSWER 26 OF 101 MEDLINE on STN

2000462123. PubMed ID: 11014174. AIDS. Escape from the immune system. **Walker B D**; Goulder P J. Nature, (2000 Sep 21) 407 (6802) 313-4. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

L10 ANSWER 27 OF 101 MEDLINE on STN

2000459408. PubMed ID: 10954555. Identification of dominant optimal HLA-B60- and HLA-B61-restricted **cytotoxic** T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. Altfeld M A; Trocha A; Eldridge R L; Rosenberg E S; Phillips M N; Addo M M; Sekaly R P; Kalams S A; Burchett S A; McIntosh K; **Walker B D**; Goulder P J. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, USA.)

Journal of virology, (2000 Sep) 74 (18) 8611-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1)-specific **cytotoxic** T-lymphocyte (CTL) responses play a major role in the **antiviral** immune response, but the relative contribution of CTL responses restricted by different HLA class I molecules is less well defined. HLA-B60 or the related allele B61 is expressed in 10 to 20% of Caucasoid populations and is even more highly prevalent in Asian populations, but yet no CTL epitopes restricted by these alleles have been defined. Here we report the definition of five novel HLA-B60-restricted **HIV**-1-specific CTL epitopes, using peripheral blood mononuclear cells in enzyme-linked immunospot (Elispot) assays and using CTL clones and lines in cytolytic assays. The dominant HLA-B60-restricted epitope, Nef peptide KEKGGLEGL, was targeted by all eight subjects with B60 and also by both subjects with B61 studied. This study additionally establishes the utility of the Elispot assay as a more rapid and efficient method of defining novel CTL epitopes. This approach will help to define new CTL epitopes that may play an important role in the immune control of **HIV**-1.

L10 ANSWER 28 OF 101 MEDLINE on STN

2000429074. PubMed ID: 10925292. Impaired CTL recognition of cells latently infected with Kaposi's sarcoma-associated herpes virus. Brander C; Suscovich T; Lee Y; Nguyen P T; O'Connor P; Seebach J; Jones N G; van Gorder M; **Walker B D**; Scadden D T. (Partners AIDS Research Center and Massachusetts General Hospital Cancer Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129, USA.) Journal of immunology (Baltimore, Md. : 1950), (2000 Aug 15) 165 (4) 2077-83. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Kaposi's sarcoma-associated herpes virus (KSHV) is a recently identified human gamma2-herpesvirus associated with Kaposi's sarcoma, primary effusion lymphoma, and Castleman's disease. We reasoned that CTL responses may provide host defense against this virus, and consequently, KSHV may have evolved strategies to evade the CTL-mediated immune surveillance. In this study six B cell lines latently infected with KSHV were found to express reduced levels of HLA class I surface molecules compared with B cell lines transformed by the related gamma-herpesvirus EBV. KSHV-infected cells also required higher concentrations of **soluble** peptides to induce efficient CTL-mediated lysis than control cell lines and were unable to process and/or present intracellularly expressed Ag. Incubation of the KSHV-infected cell lines with high concentrations of **soluble** HLA class I binding peptides did not restore the deficient HLA class I surface expression. To assess the underlying mechanisms of these phenomena, TAP-1 and TAP-2 gene expression was analyzed. While no attenuation in TAP-2 expression was observed, TAP-1 expression was significantly reduced in all KSHV cell lines compared with that in controls. These results indicate that KSHV can modulate HLA class I-restricted Ag presentation to CTL, which may allow latently infected cells to escape CTL recognition and persist in the infected host.

L10 ANSWER 29 OF 101 MEDLINE on STN

2000405830. PubMed ID: 10864688. Inhibition of **human immunodeficiency virus** type 1 replication in primary CD4(+) T lymphocytes, monocytes, and dendritic cells by **cytotoxic** T lymphocytes. Severino M E; Sipsas N V; Nguyen P T; Kalams S A; **Walker B D**; Johnson R P; Yang O O. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Charlestown, Massachusetts 02129, USA.) Journal of virology, (2000 Jul) 74 (14) 6695-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We demonstrate that **human immunodeficiency virus** type 1 (**HIV**-1)-specific **CD8**(+) **cytotoxic** T lymphocytes (CTL) suppress **HIV**-1 replication in primary lymphocytes, monocytes, and dendritic cells individually. Viral inhibition is significantly diminished in lymphocyte-dendritic cell clusters, suggesting that these clusters in vivo could be sites where viral replication is more difficult to control by CTL.

L10 ANSWER 30 OF 101 MEDLINE on STN
2000340297. PubMed ID: 10885771. Anti-**HIV** cellular immunity: recent advances towards vaccine design. Goulder P J; Rowland-Jones S L; McMichael A J; **Walker B D.** (AIDS Research Center, Massachusetts General Hospital, Boston, USA.) AIDS (London, England), (1999) 13 Suppl A S121-36. Ref: 147. Journal code: 8710219. ISSN: 0269-9370. Pub. country: ENGLAND: United Kingdom. Language: English.

L10 ANSWER 31 OF 101 MEDLINE on STN
2000283828. PubMed ID: 10823876. Differential narrow focusing of immunodominant **human immunodeficiency virus** gag-specific **cytotoxic** T-lymphocyte responses in infected African and caucasoid adults and children. Goulder P J; Brander C; Annamalai K; Mngqundaniso N; Govender U; Tang Y; He S; Hartman K E; O'Callaghan C A; Ogg G S; Altfeld M A; Rosenberg E S; Cao H; Kalams S A; Hammond M; Bunce M; Pelton S I; Burchett S A; McIntosh K; Coovadia H M; **Walker B D.** (Partners AIDS Research Center, Massachusetts General Hospital, Charlestown, Massachusetts 02129, USA.. goulder@helix.mgh.harvard.edu) . Journal of virology, (2000 Jun) 74 (12) 5679-90. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Cytotoxic** T-lymphocyte (CTL) activity plays a central role in control of viral replication and in determining outcome in cases of **human immunodeficiency virus** type 1 (**HIV**-1) infection. Incorporation of important CTL epitope sequences into candidate vaccines is, therefore, vital. Most CTL studies have focused upon small numbers of adult Caucasoid subjects infected with clade-B virus, whereas the global epidemic is most severe in sub-Saharan African populations and predominantly involves clade-C infection in both adults and children. In this study, sensitive enzyme-linked immunospot (elispot) assays have been utilized to identify the dominant Gag-specific CTL epitopes targeted by adults and children infected with clade-B or -C virus. Cohorts evaluated included 44 B-clade-infected Caucasoid American and African American adults and children and 37 C-clade-infected African adults and children from Durban, South Africa. The results show that 3 out of 46 peptides spanning p17(Gag) and p24(Gag) sequences tested contain two-thirds of the dominant Gag-specific epitopes, irrespective of the clade, ethnicity, or age group studied. However, there were distinctive differences between the dominant responses made by Caucasoids and Africans. Dominant responses in Caucasoids were more often within p17(Gag) peptide residues 16 to 30 (38 versus 12%; $P < 0.01$), while p24(Gag) peptide residues 41 to 60 contained the dominant Gag epitope more often in the African subjects tested (39 versus 4%; $P < 0.005$). Within this 20-mer p24(Gag), an epitope presented by both B42 and B81 is defined which represents the dominant Gag response in >30% of the total infected population in Durban. This epitope is closely homologous with dominant **HIV**-2 and **simian immunodeficiency virus** Gag-specific CTL epitopes. The fine focusing of dominant CTL responses to these few regions of high immunogenicity is of significance to vaccine design.

L10 ANSWER 32 OF 101 MEDLINE on STN
2000261752. PubMed ID: 10799606. HLA-B57-restricted **cytotoxic** T-lymphocyte activity in a single infected subject toward two optimal epitopes, one of which is entirely contained within the other. Goulder P J; Tang Y; Pelton S I; **Walker B D.** (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, USA.. goulder@helix.mgh.harvard.edu) . Journal of virology, (2000 Jun) 74 (11) 5291-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Viral peptides are recognized by **cytotoxic** T lymphocytes (CTL) as a complex with major histocompatibility complex (MHC) class I molecules, but the extent to which a single HLA allele can accommodate epitope peptides of different length and sequence is not well characterized. Here we report the identification of clonal CTL responses from the same donor that independently recognize one of two HLA-B57-restricted epitopes, KAFSPEVIPMF (KF11; p24(Gag) residues 30 to 40) and KAFSPEVI (KF8; p24(Gag)

peptide stabilized the HLA-B57-peptide complex more efficiently than the KI8 peptide, strong clonal responses were directed at each epitope. In samples from a second donor, the same phenomenon was observed, in which distinct CTL clones recognized peptide epitopes presented by the same HLA class I allele (in this case, HLA-A3) which were entirely overlapping. These data are relevant to the accurate characterization of CTL responses, which is fundamental to a detailed understanding of MHC class I-restricted immunity. In addition, these studies demonstrate marked differences in the length of peptides presented by HLA-B57, an allele which is associated with nonprogressive **human immunodeficiency virus** infection.

L10 ANSWER 33 OF 101 MEDLINE on STN

2000027246. PubMed ID: 10559335. Efficient processing of the immunodominant, HLA-A*0201-restricted **human immunodeficiency virus** type 1 **cytotoxic** T-lymphocyte epitope despite multiple variations in the epitope flanking sequences. Brander C; Yang O O; Jones N G; Lee Y; Goulder P; Johnson R P; Trocha A; Colbert D; Hay C; Buchbinder S; Bergmann C C; Zweerink H J; Wolinsky S; Blattner W A; Kalams S A; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, USA.. brander@helix.mgh.harvard.edu) . Journal of virology, (1999 Dec) 73 (12) 10191-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Immune escape from **cytotoxic** T-lymphocyte (CTL) responses has been shown to occur not only by changes within the targeted epitope but also by changes in the flanking sequences which interfere with the processing of the immunogenic peptide. However, the frequency of such an escape mechanism has not been determined. To investigate whether naturally occurring variations in the flanking sequences of an immunodominant **human immunodeficiency virus** type 1 (**HIV**-1) Gag CTL epitope prevent antigen processing, cells infected with **HIV**-1 or vaccinia virus constructs encoding different patient-derived Gag sequences were tested for recognition by HLA-A*0201-restricted, p17-specific CTL. We found that the immunodominant p17 epitope (SL9) and its variants were efficiently processed from minigene expressing vectors and from six **HIV**-1 Gag variants expressed by recombinant vaccinia virus constructs. Furthermore, SL9-specific CTL clones derived from multiple donors efficiently inhibited virus replication when added to HLA-A*0201-bearing cells infected with primary or laboratory-adapted strains of virus, despite the variability in the SL9 flanking sequences. These data suggest that escape from this immunodominant CTL response is not frequently accomplished by changes in the epitope flanking sequences.

L10 ANSWER 34 OF 101 MEDLINE on STN

2000015179. PubMed ID: 10545982. The great escape - AIDS viruses and immune control. Goulder P J; **Walker B D**. Nature medicine, (1999 Nov) 5 (11) 1233-5. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB Many studies have been designed to address the role of CTL immune escape in **HIV**-1 infection, but have not given conclusive answers. Now, an elegant longitudinal analysis clearly demonstrates that progression to disease in **SIV**-infected macaques is associated with evasion of the CTL response (pages 1270-1276).

L10 ANSWER 35 OF 101 MEDLINE on STN

1999379880. PubMed ID: 10448136. T lymphocyte responses in **HIV**-1 infection: implications for vaccine development. Brander C; **Walker B D**. (Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, MGH-East, 5th floor, 149 13th Street, Charlestown, MA 02129, USA.. brander@helix.mgh.harvard.edu) . Current opinion in immunology, (1999 Aug) 11 (4) 451-9. Ref: 106. Journal code: 8900118. ISSN: 0952-7915. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Substantial progress has been made over the past year in understanding the cellular immune response in **HIV** pathogenesis. **Cytotoxic** T lymphocytes play a critical role in establishing the level of viremia and

virus specific T cell responses appear to direct the in vivo efficacy of **cytotoxic** T lymphocytes. Together, these new data provide important insights to refocus efforts aimed at immunotherapeutic interventions and vaccine development.

L10 ANSWER 36 OF 101 MEDLINE on STN
1999329197. PubMed ID: 10400770. Levels of **human immunodeficiency virus** type 1-specific **cytotoxic** T-lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. Kalams S A; Goulder P J; Shea A K; Jones N G; Trocha A K; Ogg G S; **Walker B D.** (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA.. Kalams@helix.mgh.harvard.edu) . Journal of virology, (1999 Aug) 73 (8) 6721-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Therapeutic suppression of **human immunodeficiency virus** type 1 (**HIV**-1) replication may help elucidate interactions between the host cellular immune responses and **HIV**-1 infection. We performed a detailed longitudinal evaluation of two subjects before and after the start of highly active antiretroviral therapy (HAART). Both subjects had evidence of in vivo-activated and memory **cytotoxic** T-lymphocyte precursor (CTLp) activity against multiple **HIV**-1 gene products. After the start of therapy, both subjects had declines in the levels of in vivo-activated **HIV**-1-specific CTLs and had immediate increases in circulating **HIV**-1-specific CTL memory cells. With continued therapy, and continued suppression of viral load, levels of memory CTLps declined. HLA A*0201 peptide tetramer staining demonstrated that declining levels of in vivo-activated CTL activity were associated with a decrease in the expression of the CD38(+) activation marker. Transient increases in viral load during continued therapy were associated with increases in the levels of virus-specific CTLps in both individuals. The results were confirmed by measuring CTL responses to discrete optimal epitopes. These studies illustrate the dynamic equilibrium between the host immune response and levels of viral antigen burden and suggest that efforts to augment **HIV**-1-specific immune responses in subjects on HAART may decrease the incidence of virologic relapse.

L10 ANSWER 37 OF 101 MEDLINE on STN
1999329196. PubMed ID: 10400769. Association between virus-specific **cytotoxic** T-lymphocyte and helper responses in **human immunodeficiency virus** type 1 infection. Kalams S A; Buchbinder S P; Rosenberg E S; Billingsley J M; Colbert D S; Jones N G; Shea A K; Trocha A K; **Walker B D.** (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, and Harvard Medical School, Boston, Massachusetts 02114, USA.. kalams@helix.mgh.harvard.edu) . Journal of virology, (1999 Aug) 73 (8) 6715-20. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Cellular immune responses are thought to be an important **antiviral** host defense, but the relationship between virus-specific T-helper and **cytotoxic**-T-lymphocyte (CTL) responses has not been defined. To investigate a potential link between these responses, we examined functional **human immunodeficiency virus** type 1 (**HIV**-1)-specific memory CTL precursor frequencies and p24-specific proliferative responses in a cohort of infected untreated persons with a wide range of viral loads and CD4 cell counts. Levels of p24-specific proliferative responses positively correlated with levels of Gag-specific CTL precursors and negatively correlated with levels of plasma **HIV**-1 RNA. These data linking the levels of **HIV**-specific CTL with virus-specific helper cell function during chronic viral infection provide cellular immunologic parameters to guide therapeutic and prophylactic vaccine development.

L10 ANSWER 38 OF 101 MEDLINE on STN
1999292843. PubMed ID: 10364299. Lack of viral escape and defective in vivo activation of **human immunodeficiency virus** type 1-specific **cytotoxic** T lymphocytes in rapidly progressive infection. Hay C M; Ruhl

T; Wolinsky S M; Crawford J M; Montefiori D C; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.) Journal of virology, (1999 Jul) 73 (7) 5509-19. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1)-specific immune responses over the course of rapidly progressive infection are not well defined. Detailed longitudinal analyses of neutralizing antibodies, lymphocyte proliferation, in vivo-activated and memory **cytotoxic** T-lymphocyte (CTL) responses, and viral sequence variation were performed on a patient who presented with acute **HIV**-1 infection, developed an AIDS-defining illness 13 months later, and died 45 months after presentation. Neutralizing-antibody responses remained weak throughout, and no **HIV**-1-specific lymphocyte proliferative responses were seen even early in the disease course. Strong in vivo-activated CTL directed against Env and Pol epitopes were present at the time of the initial drop in viremia but were quickly lost. Memory CTL against Env and Pol epitopes were detected throughout the course of infection; however, these CTL were not activated in vivo. Despite an initially narrow CTL response, new epitopes were not targeted as the disease progressed. Viral sequencing showed the emergence of variants within the two targeted CTL epitopes; however, viral variants within the immunodominant Env epitope were well recognized by CTL, and there was no evidence of viral escape from immune system detection within this epitope. These data demonstrate a narrowly directed, static CTL response in a patient with rapidly progressive disease. We also show that disease progression can occur in the presence of persistent memory CTL recognition of autologous epitopes and in the absence of detectable escape from CTL responses, consistent with an in vivo defect in activation of CTL.

L10 ANSWER 39 OF 101 MEDLINE on STN

1999218520. PubMed ID: 10202022. Persistent **HIV**-1-specific CTL clonal expansion despite high viral burden post in utero **HIV**-1 infection. Brander C; Goulder P J; Luzuriaga K; Yang O O; Hartman K E; Jones N G; **Walker B D**; Kalams S A. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.) Journal of immunology (Baltimore, Md. : 1950), (1999 Apr 15) 162 (8) 4796-800. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB To address the issue of clonal exhaustion in humans, we monitored HLA class I-restricted, epitope-specific CTL responses in an in utero **HIV**-1-infected infant from 3 mo through 5 years of age. Serial functional CTL precursor assays demonstrated persistent, vigorous, and broadly directed **HIV**-1 specific CTL activity with a dominant response against an epitope in **HIV**-1 Gag-p17 (SLYNTVATL, aa 77-85). A clonal CTL response directed against the immunodominant, HLA-A*0201-restricted epitope was found to persist over the entire observation period, as shown by TCR analysis of cDNA libraries generated from PBMC. The analysis of autologous viral sequences did not reveal any escape mutations within the targeted epitope, and viral load measurement indicated ongoing viral replication. Furthermore, inhibition of viral replication assays indicated that the epitope was properly processed from autologous viral protein. These data demonstrate that persistent exposure to high levels of viral Ag does not necessarily lead to clonal exhaustion and that epitope-specific clonal CTL responses induced within the first weeks of life can persist for years without inducing detectable viral escape variants.

L10 ANSWER 40 OF 101 MEDLINE on STN

1999217689. PubMed ID: 10203039. Characterization of **HIV**-1-specific T-helper cells in acute and chronic infection. Rosenberg E S; LaRosa L; Flynn T; Robbins G; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston 02114, USA.) Immunology letters, (1999 Mar) 66 (1-3) 89-93. Journal code: 7910006. ISSN: 0165-2478. Pub. country:

Netherlands. Language: English.

AB **HIV**-1 infection is associated with progressive and relentless destruction of the immune system in the majority of infected persons, but some persons appear to be able to successfully contain the virus in the absence of **antiviral** therapy. Such cases suggest that the host immune response can successfully contain the virus, and provide the rationale for concerted efforts to understand the host immune response to the virus and to develop new strategies to combat the infection with immune based therapies. Historically, the greatest hole in the immune repertoire in **HIV**-1 infection has been the lack of strong virus-specific proliferative responses. However, new studies have identified a potent Th cell response in some infected persons, and have shown a statistically significant negative correlation between plasma viremia and virus-specific CD4 T-helper cells directed at the p24 protein. Moreover, early institution of potent **antiviral** therapy in the earliest stages of acute **HIV**-1 infection have led to persistent, strong **HIV**-1-specific T-helper cell responses, analogous to those seen in persons who are able to control viremia in the absence of **antiviral** therapy. We hypothesize that this is because potent **antiviral** therapy is able to protect virus-specific Th cells as they become activated, and thus these cells are not lost in the earliest stages of infection.

L10 ANSWER 41 OF 101 MEDLINE on STN

1999214336. PubMed ID: 10196293. Frequent detection of escape from **cytotoxic** T-lymphocyte recognition in perinatal **human immunodeficiency virus** (**HIV**) type 1 transmission: the ariel project for the prevention of transmission of **HIV** from mother to infant. Wilson C C; Brown R C; Korber B T; Wilkes B M; Ruhl D J; Sakamoto D; Kunstman K; Luzuriaga K; Hanson I C; Widmayer S M; Wiznia A; Clapp S; Ammann A J; Koup R A; Wolinsky S M; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA.) Journal of virology, (1999 May) 73 (5) 3975-85. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Host immunologic factors, including **human immunodeficiency virus** (**HIV**)-specific **cytotoxic** T lymphocytes (CTL), are thought to contribute to the control of **HIV** type 1 (**HIV**-1) replication and thus delay disease progression in infected individuals. Host immunologic factors are also likely to influence perinatal transmission of **HIV**-1 from infected mother to infant. In this study, the potential role of CTL in modulating **HIV**-1 transmission from mother to infant was examined in 11 **HIV**-1-infected mothers, 3 of whom transmitted virus to their offspring. Frequencies of **HIV**-1-specific human leukocyte antigen class I-restricted CTL responses and viral epitope amino acid sequence variation were determined in the mothers and their infected infants. Maternal **HIV**-1-specific CTL clones were derived from each of the **HIV**-1-infected pregnant women. Amino acid substitutions within the targeted CTL epitopes were more frequently identified in transmitting mothers than in nontransmitting mothers, and immune escape from CTL recognition was detected in all three transmitting mothers but in only one of eight nontransmitting mothers. The majority of viral sequences obtained from the **HIV**-1-infected infant blood samples were susceptible to maternal CTL. These findings demonstrate that epitope amino acid sequence variation and escape from CTL recognition occur more frequently in mothers that transmit **HIV**-1 to their infants than in those who do not. However, the transmitted virus can be a CTL susceptible form, suggesting inadequate in vivo immune control.

L10 ANSWER 42 OF 101 MEDLINE on STN

1999192796. PubMed ID: 10092836. Molecular and functional analysis of a conserved CTL epitope in **HIV**-1 p24 recognized from a long-term nonprogressor: constraints on immune escape associated with targeting a sequence essential for viral replication. Wagner R; Leschonsky B; Harrer E; Paulus C; Weber C; **Walker B D**; Buchbinder S; Wolf H; Kalden J R; Harrer T. (Institute of Medical Microbiology, University of Regensburg, Germany.) Journal of immunology (Baltimore, Md. : 1950), (1999 Mar 15)

United States. Language: English.

AB It has been hypothesized that sequence variation within CTL epitopes leading to immune escape plays a role in the progression of **HIV**-1 infection. Only very limited data exist that address the influence of biologic characteristics of CTL epitopes on the emergence of immune escape variants and the efficiency of suppression **HIV**-1 by CTL. In this report, we studied the effects of **HIV**-1 CTL epitope sequence variation on **HIV**-1 replication. The highly conserved HLA-B14-restricted CTL epitope DRFYKTLRAE in **HIV**-1 p24 was examined, which had been defined as the immunodominant CTL epitope in a long-term nonprogressing individual. We generated a set of viral mutants on an HX10 background differing by a single conservative or nonconservative amino acid substitution at each of the P1 to P9 amino acid residues of the epitope. All of the nonconservative amino acid substitutions abolished viral infectivity and only 5 of 10 conservative changes yielded replication-competent virus. Recognition of these epitope sequence variants by CTL was tested using synthetic peptides. All mutations that abrogated CTL recognition strongly impaired viral replication, and all replication-competent viral variants were recognized by CTL, although some variants with a lower efficiency. Our data indicate that this CTL epitope is located within a viral sequence essential for viral replication. Targeting CTL epitopes within functionally important regions of the **HIV**-1 genome could limit the chance of immune evasion.

L10 ANSWER 43 OF 101 MEDLINE on STN

1999106643. PubMed ID: 9889969. Immune control of **HIV**-1 replication. **Walker B D**; Rosenberg E S; Hay C M; Basgoz N; Yang O O. (Partners AIDS Research Center, Massachusetts General Hospital, Charlestown 02129, USA.. bwalker@helix.mgh.harvard.edu) . Advances in experimental medicine and biology, (1998) 452 159-67. Ref: 32. Journal code: 0121103. ISSN: 0065-2598. Pub. country: United States. Language: English.

L10 ANSWER 44 OF 101 MEDLINE on STN

1999077904. PubMed ID: 9858506. The critical need for CD4 help in maintaining effective **cytotoxic** T lymphocyte responses. Kalams S A; **Walker B D**. (Partners AIDS Research Center and Infectious Disease Division, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129,. USA.kalams@helix.mgh.harvard.edu) . Journal of experimental medicine, (1998 Dec 21) 188 (12) 2199-204. Ref: 51. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

L10 ANSWER 45 OF 101 MEDLINE on STN

1999008552. PubMed ID: 9794421. Recognition of two overlapping CTL epitopes in **HIV**-1 p17 by CTL from a long-term nonprogressing **HIV**-1-infected individual. Harrer T; Harrer E; Barbosa P; Kaufmann F; Wagner R; Bruggemann S; Kalden J R; Feinberg M; Johnson R P; Buchbinder S; **Walker B D**. (Department of Medicine III with Institute of Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany.. Thomas.Harrer@med3.med.uni-erlangen.de) . Journal of immunology (Baltimore, Md. : 1950), (1998 Nov 1) 161 (9) 4875-81. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB **HIV**-1 infection has been shown to elicit strong CTL responses in some infected persons, but few data are available regarding the relationship between targeted epitopes and in vivo viral quasispecies. In this study, we examined the CTL response in a person infected for 15 yr with a CD4 count persistently >500 cells/microl. The dominant in vivo activated CTL response was directed against two overlapping Gag CTL epitopes in an area of p17 known to be essential for viral replication. The 9-mer SLYNTVATL (amino acids 77-85) was recognized in conjunction with HLA-A2, whereas the overlapping 8-mer TLYCVHQR (amino acids 83-91) was recognized by HLA-A11-restricted CTL. Analysis of in vivo virus sequences both in PBMC and plasma revealed the existence of sequence variation in this region, which did not affect viral replication in vitro, but decreased recognition by the A11-restricted CTL response, with maintenance of the A2-restricted

response. These results indicate that an essential region of the p17 protein can be simultaneously targeted by CTL through two different HLA molecules, and that immune escape from CTL recognition can occur without impairing viral replication. In addition, they demonstrate that Ag processing can allow for presentation of overlapping epitopes in the same infected cell, which can be affected quite differently by sequence variation.

L10 ANSWER 46 OF 101 MEDLINE on STN
1998453447. PubMed ID: 9780251. Longitudinal and cross-sectional analysis of **cytotoxic** T lymphocyte responses and their relationship to vertical **human immunodeficiency virus** transmission. ARIEL Project Investigators. Jin X; Roberts C G; Nixon D F; Cao Y; Ho D D; **Walker B D**; Muldoon M; Korber B T; Koup R A. (Aaron Diamond Aids Research Center, Rockfeller University, New York, NY, USA.) Journal of infectious diseases, (1998 Nov) 178 (5) 1317-26. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.
AB The ARIEL Project for the Prevention of **HIV** Transmission from Mother to Infant was established to evaluate virologic and immunologic parameters during vertical transmission. To determine the strength and breadth of the **cytotoxic** T lymphocyte (CTL) response and its correlation with **human immunodeficiency virus** (**HIV**) transmission, a cross-sectional study was done of 31 **HIV**-infected pregnant women, of whom 15 transmitted and 16 did not transmit **HIV** to their infants. The precursor frequencies of CTL specific for **HIV**-1 gag, pol, nef, and env from 5 different isolates of the clade B of **HIV**-1 were determined by limiting dilution analysis. Results showed that variable levels of **HIV**-specific CTL response were present in **HIV**-infected pregnant women during and after pregnancy. In addition, CTL precursor frequencies specific for pol and nef were higher during pregnancy in nontransmitters than in transmitters. Thus, CTL responding to different **HIV** antigens may not be contributing equally to the prevention of vertical transmission.

L10 ANSWER 47 OF 101 MEDLINE on STN
1998282291. PubMed ID: 9616227. Lack of strong immune selection pressure by the immunodominant, HLA-A*0201-restricted **cytotoxic** T lymphocyte response in chronic **human immunodeficiency virus**-1 infection. Brander C; Hartman K E; Trocha A K; Jones N G; Johnson R P; Korber B; Wentworth P; Buchbinder S P; Wolinsky S; **Walker B D**; Kalams S A. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA.) Journal of clinical investigation, (1998 Jun 1) 101 (11) 2559-66. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Despite detailed analysis of the **HIV**-1-specific **cytotoxic** T lymphocyte response by various groups, its relation to viral load and viral sequence variation remains controversial. We analyzed HLA-A*0201 restricted **cytotoxic** T lymphocyte responses in 17 **HIV**-1-infected individuals with viral loads ranging from < 400 to 221,000 **HIV** RNA molecules per milliliter of plasma. In 13 out of 17 infected subjects, CTL responses against the SLYNTVATL epitope (p17 Gag; aa 77-85) were detectable, whereas two other HLA-A*0201 restricted epitopes (ILKEPVHGV, IV9; and VIYQYMDDL, VL9) were only recognized by six and five individuals out of 17 individuals tested, respectively. Naturally occurring variants of the SL9 epitope were tested for binding to HLA-A*0201 and for recognition by specific T cell clones generated from five individuals. Although these variants were widely recognized, they differed by up to 10,000-fold in terms of variant peptide concentrations required for lysis of target cells. A comparison of viral sequences derived from 10 HLA-A*0201-positive individuals to sequences obtained from 11 HLA-A*0201-negative individuals demonstrated only weak evidence for immune selective pressure and thus question the in vivo efficacy of immunodominant CTL responses present during chronic **HIV**-1 infection.

L10 ANSWER 48 OF 101 MEDLINE on STN
1998154733. PubMed ID: 9495345. Beta-chemokines are released from

Wagner L; Yang O O; Garcia-Zepeda E A; Ge Y; Kalams S A; **Walker B D**; Pasternack M S; Luster A D. (Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown 02129, USA.) Nature, (1998 Feb 26) 391 (6670) 908-11. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **CD8**+ lymphocytes are believed to be important in host defence against the **human immunodeficiency virus** (**HIV**)-1, inhibiting **HIV**-1 replication through both cytolytic and non-cytolytic pathways. The cytolytic pathway involves calcium-dependent exocytosis of perforin and granzyme proteases, as well as Fas-mediated programmed cell death, whereas the noncytolytic pathway involves the release of chemokines that prevent viral entry. Using granzyme A as a marker of cytolytic granule proteins, and macrophage inflammatory protein (MIP)-1alpha and RANTES as markers of **HIV**-1 inhibitory chemokines, we show that these two very different mediators of viral inhibition are both localized in the cytolytic granules of **HIV**-1-specific **CD8**+ **cytotoxic** T lymphocytes (CTL). Following antigen-specific activation, these mediators are secreted together, facilitating both lysis of virion-producing cells and the inhibition of free virus. In addition, RANTES, MIP-1alpha and MIP-1beta are secreted by CTL as a macromolecular complex containing sulphated proteoglycans. This association appears to have a functional significance, because heparan sulphate facilitates RANTES inhibition of **HIV**-1 infection of monocytes.

L10 ANSWER 49 OF 101 MEDLINE on STN

1998111233. PubMed ID: 9450757. **HIV**-1 Nef protein protects infected primary cells against killing by **cytotoxic** T lymphocytes. Collins K L; Chen B K; Kalams S A; **Walker B D**; Baltimore D. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139, USA.) Nature, (1998 Jan 22) 391 (6665) 397-401. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Cytotoxic** T lymphocytes (CTLs) lyse virally infected cells that display viral peptide epitopes in association with major histocompatibility complex (MHC) class I molecules on the cell surface. However, despite a strong CTL response directed against viral epitopes, untreated people infected with the **human immunodeficiency virus** (**HIV**-1) develop AIDS. To resolve this enigma, we have examined the ability of CTLs to recognize and kill infected primary T lymphocytes. We found that CTLs inefficiently lysed primary cells infected with **HIV**-1 if the viral nef gene product was expressed. Resistance of infected cells to CTL killing correlated with nef-mediated downregulation of MHC class I and could be overcome by adding an excess of the relevant **HIV**-1 epitope as **soluble** peptide. Thus, Nef protected infected cells by reducing the epitope density on their surface. This effect of nef may allow evasion of CTL lysis by **HIV**-1-infected cells.

L10 ANSWER 50 OF 101 MEDLINE on STN

1998105804. PubMed ID: 9445059. Immunological and virological analyses of persons infected by **human immunodeficiency virus** type 1 while participating in trials of recombinant gp120 subunit vaccines. Connor R I; Korber B T; Graham B S; Hahn B H; Ho D D; **Walker B D**; Neumann A U; Vermund S H; Mestecky J; Jackson S; Fenamore E; Cao Y; Gao F; Kalams S; Kunstman K J; McDonald D; McWilliams N; Trkola A; Moore J P; Wolinsky S M. (The Aaron Diamond AIDS Research Center, The Rockefeller University, New York, New York 10016, USA.) Journal of virology, (1998 Feb) 72 (2) 1552-76. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have studied 18 participants in phase I/II clinical trials of recombinant gp120 (rgp120) subunit vaccines (MN and SF-2) who became infected with **human immunodeficiency virus** type 1 (**HIV**-1) during the course of the trials. Of the 18 individuals, 2 had received a placebo vaccine, 9 had been immunized with MN rgp120, and seven had been immunized with SF-2 rgp120. Thirteen of the 18 infected vaccinees had received three or four immunizations prior to becoming infected. Of these, two were placebo recipients, six had received MN rgp120, and five had received SF-2 rgp120. Only 1 of the 11 rgp120 recipients who had multiple

immunizations failed to develop a lasting immunoglobulin G antibody response to the immunogen. However, the antibody response to rgp120 was transient, typically having a half-life of 40 to 60 days. No significant neutralizing activity against the infecting strain was detected in any of the infected individuals at any time prior to infection. Antibody titers in subjects infected despite vaccination and in noninfected subjects were not significantly different. Envelope-specific **cytotoxic** T-lymphocyte responses measured after infection were infrequent and weak in the nine vaccinees who were tested. **HIV**-1 was isolated successfully from all 18 individuals. Sixteen of these strains had a non-syncytium-inducing (NSI) phenotype, while two had a syncytium-inducing (SI) phenotype. NSI strains used the CCR5 coreceptor to enter CD4+ cells, while an SI strain from one of the vaccinees also used CXCR4. Viruses isolated from the blood of rgp120 vaccinees were indistinguishable from viruses isolated from control individuals in terms of their inherent sensitivity to neutralization by specific monoclonal antibodies and their replication rates in vitro. Furthermore, genetic sequencing of the env genes of strains infecting the vaccinees did not reveal any features that clearly distinguished these viruses from contemporary clade B viruses circulating in the United States. Thus, despite rigorous genetic analyses, using various breakdowns of the data sets, we could find no evidence that rgp120 vaccination exerted selection pressure on the infecting **HIV**-1 strains. The viral burdens in the infected rgp120 vaccine recipients were also determined, and they were found to be not significantly different from those in cohorts of placebo-vaccinated and nonvaccinated individuals. In summary, we conclude that vaccination with rgp120 has had,to date, no obvious beneficial or adverse effects on the individuals we have studied.

L10 ANSWER 51 OF 101 MEDLINE on STN

1998035780. PubMed ID: 9367954. Vigorous **HIV**-1-specific CD4+ T cell responses associated with control of viremia. Rosenberg E S; Billingsley J M; Caliendo A M; Boswell S L; Sax P E; Kalams S A; **Walker B D.** (Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, and Harvard Medical School, Boston, MA 02114, USA.) Science, (1997 Nov 21) 278 (5342) 1447-50. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Virus-specific CD4+ T helper lymphocytes are critical to the maintenance of effective immunity in a number of chronic viral infections, but are characteristically undetectable in chronic **human immunodeficiency virus**-type 1 (**HIV**-1) infection. In individuals who control viremia in the absence of **antiviral** therapy, polyclonal, persistent, and vigorous **HIV**-1-specific CD4+ T cell proliferative responses were present, resulting in the elaboration of interferon-gamma and **antiviral** beta chemokines. In persons with chronic infection, **HIV**-1-specific proliferative responses to p24 were inversely related to viral load. Strong **HIV**-1-specific proliferative responses were also detected following treatment of acutely infected persons with potent **antiviral** therapy. The **HIV**-1-specific helper cells are likely to be important in immunotherapeutic interventions and vaccine development.

L10 ANSWER 52 OF 101 MEDLINE on STN

1998001384. PubMed ID: 9343219. **Cytotoxic** T-lymphocyte cross-reactivity among different **human immunodeficiency virus** type 1 clades: implications for vaccine development. Cao H; Kanki P; Sankale J L; Dieng-Sarr A; Mazzara G P; Kalams S A; Korber B; Mboup S; **Walker B D.** (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Boston 02114, USA.) Journal of virology, (1997 Nov) 71 (11) 8615-23. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Despite recent advances in **antiviral** therapy for **human immunodeficiency virus** (**HIV**) infection, successful global intervention will require an effective vaccine. Expanding evidence suggests that **cytotoxic** T-lymphocyte (CTL) responses will be an important component of such a vaccine. The varying geographic distribution of **HIV** type 1 (**HIV**-1) clades, with the relative absence of clade B **HIV**-1 outside the developed world, is considered a major

obstacle to the development of a single effective vaccine. An understanding of cross-reactive CTL responses between different **HIV**-1 clades is crucial in the design of a vaccine which will be broadly immunogenic. In this study, we examined the ability of **HIV**-1 Gag-, reverse transcriptase-, and Env-specific CTL clones isolated from individuals infected in the United States to recognize non-B clade viral sequences and found that all were cross-reactive with the majority of non-B clade viral sequences tested. We next studied **HIV**-1-specific CTL responses in African individuals infected with clade A, C, or G virus and evaluated cross-recognition of clade B virus. Of 14 persons evaluated, all demonstrated cross-reactivity with the U.S. clade B viral constructs. We conclude that significant CTL cross-reactivity exists between clade B and non-B epitopes, suggesting that CTL cross-recognition among **HIV**-1 clades is more widespread than anticipated and that a vaccine based on a single clade may be broadly applicable.

L10 ANSWER 53 OF 101 MEDLINE on STN

97470992. PubMed ID: 9326635. Lysis of **HIV**-1-infected cells and inhibition of viral replication by universal receptor T cells. Yang O O; Tran A C; Kalams S A; Johnson R P; Roberts M R; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA.) Proceedings of the National Academy of Sciences of the United States of America, (1997 Oct 14) 94 (21) 11478-83. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Increasing evidence suggests that **HIV**-1-specific **cytotoxic** T lymphocytes (CTLs) are a key host immune response to **HIV**-1 infection. Generation of CTL responses for prevention or therapy of **HIV**-1 infection has several intrinsic technical barriers such as antigen expression and presentation, the varying HLA restrictions between different individuals, and the potential for viral escape by sequence variation or surface molecule alteration on infected cells. A strategy to circumvent these limitations is the construction of a chimeric T cell receptor containing human CD4 or **HIV**-1-specific Ig sequences linked to the signaling domain of the T cell receptor zeta chain (universal T cell receptor). **CD8**+ CTLs transduced with this universal receptor can then bind and lyse infected cells that express surface **HIV**-1 gp120. We evaluated the ability of universal-receptor-bearing **CD8**+ cells from a seronegative donor to lyse acutely infected cells and inhibit **HIV**-1 replication in vitro. The kinetics of lysis and efficiency of inhibition were comparable to that of naturally occurring **HIV**-1-specific CTL clones isolated from infected individuals. Further study will be required to determine the utility of these cells as a therapeutic strategy in vivo.

L10 ANSWER 54 OF 101 MEDLINE on STN

97469172. PubMed ID: 9328644. **CD8**+ cells in **human immunodeficiency virus** type I pathogenesis: cytolytic and noncytolytic inhibition of viral replication. Yang O O; **Walker B D**. (AIDS Research Center, Massachusetts General Hospital, Boston 02114, USA.) Advances in immunology, (1997) 66 273-311. Ref: 205. Journal code: 0370425. ISSN: 0065-2776. Pub. country: United States. Language: English.

L10 ANSWER 55 OF 101 MEDLINE on STN

97411686. PubMed ID: 9266632. Characteristics of the intrahepatic **cytotoxic** T lymphocyte response in chronic hepatitis C virus infection. Koziel M J; **Walker B D**. (Infectious Disease Division, Beth Israel Deaconess Medical Ctr., Boston, MA 02215, USA.) Springer seminars in immunopathology, (1997) 19 (1) 69-83. Ref: 90. Journal code: 7910384. ISSN: 0344-4325. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Based on our CTL studies of over 44 persons with chronic HCV infection, we are able to arrive at a number of conclusions. Clearly this cellular immune response is heterogeneous among infected persons. We have not identified any specific HCV protein which appears to be immunodominant for CTL responses, but rather we have detected diverse responses to both structural and non-structural proteins. Using an identical stimulation

strategy for all persons studied, we have been able to detect responses in only approximately one third of persons with chronic infection. Among these persons, the responses among liver-infiltrating lymphocytes are greater than those detected in fresh peripheral blood, suggesting that the CTL are homing to the site of maximal viral burden in these persons. Some viral proteins contain overlapping epitopes presented by more than one HLA class I molecule, and we have also found cases where peptides in the same HLA superfamily, such as the HLA A3 superfamily which contains A11, for which the same peptide can be presented by both alleles (manuscript in preparation). Although sequence variation between the infecting strain and the vaccinia constructs used to test for responses may lead to non-recognition of some variants, even the highly conserved core protein appears to be an inconsistent and actually infrequent target for detectable CTL responses. The magnitude of the CTL response appears to vary greatly, from being undetectable to being so vigorous that it an be detected in stimulated peripheral blood. The breadth of the response also varies widely, ranging from the detection of a response to a single epitope in some persons, to the simultaneous recognition of up to five different epitopes in others. Even in persons of the same HLA type, we have not seen consistent targeting of the same epitopes except in rare cases. Despite the detection of over 20 epitopes and their restricting class I alleles using CTR derived from liver-infiltrating lymphocytes, we have identified only one epitope that has been shown to be targeted by more than one person of the same HLA type. These findings lead us to speculate that the CTL response may be submaximal in the majority of infected persons. The reasons for this are presently obscure, but could relate to a number of factors. The epitopes targeted are found within variable regions of the virus, such that immune escape from established CTL responses has to be considered a real possibility. Sequence variation may also lead to antagonism of CTL responses, as has been demonstrated for both **HIV** and HBV infections. Furthermore, sequence variation either within or adjacent to regions containing CTL epitopes can lead to altered antigen processing, either due to alteration of proteolytic processing of the viral peptides in the cytoplasm or to altered transport and altered association with class I molecules. A number of issues regarding the CTL response in HCV infection still require substantial attention. The apparent inability of CTL to clear this virus needs to be addressed, as does the potential role for viral immunomodulatory molecules in HCV persistence. Although we and others have shown CTL responses to be present in persons with chronic infection, the role of CTL in acute HCV infection needs to be determined. The best studied chronic human viral infection is **HIV** infection, in which expanding data indicate that the early events following primary infection predict the subsequent course of illness. Viral load in the first 1-2 years after infection is highly predictive of the subsequent disease course in **HIV** infection, and recent experimental data in humans suggest that early immune responses may be predictive of subsequent disease course. Such studies in HCV infection have been difficult to achieve, since primary HCV infection is often asymptomatic, and transfusion-related cases are now rare. (ABSTRACT TRUNCATED)

L10 ANSWER 56 OF 101 MEDLINE on STN

97400330. PubMed ID: 9257824. Degenerate and promiscuous recognition by CTL of peptides presented by the MHC class I A3-like superfamily: implications for vaccine development. Threlkeld S C; Wentworth P A; Kalams S A; Wilkes B M; Ruhl D J; Keogh E; Sidney J; Southwood S; **Walker B D**; Sette A. (Department of Immunology, Cytel Corporation, San Diego, CA 92121, USA.) Journal of immunology (Baltimore, Md. : 1950), (1997 Aug 15) 159 (4) 1648-57. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Recent data demonstrate that HLA class I alleles can be grouped into superfamilies based on similarities of their peptide-binding motifs. In this study, we have tested the immunogenicity and antigenicity of peptides capable of degenerate binding to multiple HLA class I molecules of the A3-like superfamily. The assay systems utilized included both primary in vitro cultures of lymphocytes from healthy donors, as well as in vitro

[illegible] lymphocytes from HIV infected individuals. Several of the peptides capable of binding more than one HLA A3-like class I molecule were also found to be immunogenic in the context of this same group of A3-like molecules (degenerate CTL recognition). Furthermore, some of the CTL lines thus generated demonstrated promiscuous recognition of the cognate epitope in the context of MHC molecules from more than one member of the superfamily. The fine Ag specificity of this phenomenon was further analyzed using two promiscuous CTL clones derived from A3 and A11 individuals, respectively, and specific for an epitope in the **HIV**-1 reverse transcriptase. By the use of single-amino acid-substitution analogues, it was demonstrated that the fine specificity of the TCR is largely maintained between MHC-matched and MHC-mismatched presentation of peptide within the A3-like superfamily. These results indicate that the similar peptide-binding specificities among different members of the A3-like superfamily can be reflected in a remarkable similarity in the peptide-MHC complex structures engaged by the TCR and responsible for T cell activation.

L10 ANSWER 57 OF 101 MEDLINE on STN

97245194. PubMed ID: 9089953. **HIV**-1-specific **cytotoxic** T lymphocytes and the control of **HIV**-1 replication. Jassoy C; **Walker B D**. (Institute for Virology and Immunobiology, Julius-Maximilians University, Wurzburg, Germany.) Springer seminars in immunopathology, (1997) 18 (3) 341-54. Ref: 102. Journal code: 7910384. ISSN: 0344-4325. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L10 ANSWER 58 OF 101 MEDLINE on STN

97244174. PubMed ID: 9088983. Stimulation of human **cytotoxic** T cells with **HIV**-1-derived peptides presented by recombinant HLA-A2 peptide complexes. Walter J B; Brander C; Mammen M; Garboczi D N; Kalams S A; Whitesides G M; **Walker B D**; Eisen H N. (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139, USA.) International immunology, (1997 Mar) 9 (3) 451-9. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB HLA-A2 heavy chain and beta 2-microglobulin were expressed in Escherichia coli, and refolded in the presence of peptides derived from **HIV**-1 RT and gag proteins. When recombinant HLA-A2 molecules were attached to cells lacking HLA-A2, the cells became susceptible to lysis by HLA-A2-restricted **cytotoxic** T lymphocyte (CTL) clones specific for peptides derived from RT and gag proteins. Limiting dilution analyses of peripheral blood mononuclear cells from **HIV**-1-infected individuals showed that the recombinant HLA-A2 peptide complexes covalently immobilized on microspheres stimulated the development of HLA-A2 peptide-specific CTL. Preformed HLA-peptide complexes may provide an alternative to immunization procedures that depend upon intracellular processing of antigen to elicit T cell responses.

L10 ANSWER 59 OF 101 MEDLINE on STN

97213986. PubMed ID: 9060675. Suppression of **human immunodeficiency virus** type 1 replication by **CD8**+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. Yang O O; Kalams S A; Trocha A; Cao H; Luster A; Johnson R P; **Walker B D**. (AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown 02129, USA.) Journal of virology, (1997 Apr) 71 (4) 3120-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Although **CD8**+ lymphocytes in **human immunodeficiency virus** type 1 (**HIV**-1)-infected individuals have been demonstrated to suppress viral replication, the mechanisms of inhibition have not been defined precisely. A large body of evidence indicates that these cells act via **soluble** inhibitory factors, but the potential role of HLA class I-restricted cytolysis has remained controversial. Here we demonstrate that **HIV**-1-specific **cytotoxic** T lymphocytes (CTL) mediate **antiviral** suppression by both cytolytic and noncytolytic mechanisms. The predominant mechanism requires direct contact of CTL with the infected cells, is HLA class I restricted, and can achieve complete elimination of

detectable virus in infected cell cultures. Inhibition occurs even at high multiplicities of infection or at ratios of CTL to CD4 cells as low as 1:1,000. The other mechanism is mediated by **soluble** inhibitory factors which are triggered in an antigen-specific and HLA-restricted fashion but then act without HLA restriction. These include MIP-1alpha, MIP-1beta, and RANTES, as well as a distinct factor(s) capable of inhibiting **HIV**-1 strains insensitive to these chemokines. These data indicate that **HIV**-1-specific CTL are potent mediators of **HIV**-1 suppression at cell ratios existing in vivo and demonstrate an antigen-specific trigger for **CD8**+ cell-derived **soluble** inhibitory factors. These results suggest that CTL play an important role in the observed **antiviral** activity of **CD8**+ cells from infected individuals.

L10 ANSWER 60 OF 101 MEDLINE on STN

97197584. PubMed ID: 9045880. Identification of type-specific **cytotoxic** T lymphocyte responses to homologous viral proteins in laboratory workers accidentally infected with **HIV**-1. Sipsas N V; Kalams S A; Trocha A; He S; Blattner W A; **Walker B D**; Johnson R P. (AIDS Research Center, Infectious Disease Unit, Massachusetts General Hospital, Charlestown 02129, USA.) Journal of clinical investigation, (1997 Feb 15) 99 (4) 752-62. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Characterization of the **cytotoxic** T lymphocyte (CTL) response against **HIV**-1 has been limited by the use of target cells expressing viral proteins from laboratory isolates of **HIV**-1. This approach has favored identification of group-specific CTL responses and precluded assessment of the extent of type-specific CTL responses directed against **HIV**-1. Using cells expressing viral proteins from the **HIV**-1 IIIB strain, we performed a detailed characterization of **HIV**-1-specific CTL response in three laboratory workers accidentally infected with **HIV**-1 IIIB. Eight of the epitopes identified were group specific, lying in relatively conserved regions of Gag, reverse transcriptase, and envelope. Three type-specific epitopes were identified, two of them in highly variable regions of envelope. In longitudinal studies in one subject, seven different epitopes and five different restricting HLA class I alleles were identified, with a progressive increase in the number of CTL epitopes recognized by this subject over time. Our data demonstrate that type-specific CTL responses make up a significant proportion of the host cellular immune response against **HIV**-1 and that a broadening of epitope specificity may occur.

L10 ANSWER 61 OF 101 MEDLINE on STN

97164825. PubMed ID: 9012587. T lymphocytes and their cytokines in **human immunodeficiency virus** (**HIV**) infection: implications for associated neoplasias. Jassoy C; **Walker B D**. (Institute for Virology and Immunobiology, Wurzburg University, Germany.) Critical reviews in oncogenesis, (1995) 6 (3-6) 275-89. Ref: 143. Journal code: 8914610. ISSN: 0893-9675. Pub. country: United States. Language: English.

AB Infection with the **human immunodeficiency virus** (**HIV**) results in gradual immunosuppression due to the loss of CD4+ T cells. In the wake of immune system breakdown, infected individuals may acquire multiple opportunistic infections and develop certain malignancies which ultimately account for the vast majority of deaths in these persons. A limited number of malignancies are directly associated with **HIV** infection and suggest a common tie between these tumors. Inappropriate immune surveillance resulting in insufficient inhibition of virus replication and inadequate control of the growth of transformed cells may contribute to the development of malignancies in **HIV**-infected individuals. Alternatively, malignancies in **HIV** infection may be the consequence of immune dysregulation. Cellular immune responses mediated by antigen-specific **cytotoxic** T lymphocytes (CTL) are of particular importance for immunologic control of viral infections and substantial information has been gathered about theses cells in **HIV** infection. The goal of this review is therefore to summarize recent findings regarding the cellular immune response to **HIV** with a particular focus on cytokines released by **HIV**-specific CTL.

L10 ANSWER 62 OF 101 MEDLINE on STN

97151113. PubMed ID: 8995649. Overlapping epitopes in **human immunodeficiency virus** type 1 gp120 presented by HLA A, B, and C molecules: effects of viral variation on **cytotoxic** T-lymphocyte recognition. Wilson C C; Kalams S A; Wilkes B M; Ruhl D J; Gao F; Hahn B H; Hanson I C; Luzuriaga K; Wolinsky S; Koup R; Buchbinder S P; Johnson R P; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Boston 02114, USA.) Journal of virology, (1997 Feb) 71 (2) 1256-64. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** (**HIV**)-specific **cytotoxic** T lymphocytes (CTL) are thought to exert immunologic selection pressure in infected persons, yet few data regarding the effects of this constraint on viral sequence variation in vivo, particularly in the highly variable Env protein, are available. In this study, **CD8+ HIV** type 1 (**HIV**-1) envelope-specific CTL clones specific for gp120 were isolated from peripheral blood mononuclear cells of four **HIV**-infected individuals, all of which recognized the same 25-amino-acid (aa) peptide (aa 371 to 395), which is partially contained in the CD4-binding domain of **HIV**-1 gp120. Fine mapping studies revealed that two of the clones optimally recognized the 9-aa sequence 375 to 383 (SFNCGGEFF), while the two other clones optimally recognized the epitope contained in the overlapping 9-aa sequence 376 to 384 (FNCGGEFFY). Lysis of target cells by the two clones recognizing aa 375 to 383 was restricted by HLA B15 and Cw4, respectively, whereas both clones recognizing aa 376 to 384 were restricted by HLA A29. Sequence variation, relative to the IIIB strain sequence used to identify CTL clones, was observed in autologous viruses in the epitope-containing region in all four subjects. However, poorly recognized autologous sequence variants were predominantly seen for the A29-restricted clones, whereas the clones specific for SFNCGGEFF continued to recognize the predominant autologous sequences. These results suggest that the HLA profile of an individual may not only be important in determining the specificity of CTL recognition but may also affect the ability to recognize virus variants and suppress escape from CTL recognition. These results also identify overlapping viral CTL epitopes which can be presented by HLA A, B, and C molecules.

L10 ANSWER 63 OF 101 MEDLINE on STN

96323090. PubMed ID: 8709196. Efficient lysis of **human immunodeficiency virus** type 1-infected cells by **cytotoxic** T lymphocytes. Yang O O; Kalams S A; Rosenzweig M; Trocha A; Jones N; Koziel M; **Walker B D**; Johnson R P. (AIDS Research Center, Massachusetts General Hospital, Charlestown 02129, USA.) Journal of virology, (1996 Sep) 70 (9) 5799-806. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Numerous studies of **human immunodeficiency virus** type 1 (**HIV**-1)-specific **cytotoxic** T lymphocytes (CTL) have examined their ability to recognize B-cell lines expressing recombinant **HIV**-1 proteins, but relatively few data regarding the lysis of **HIV**-1-infected cells by CTL are available. We studied the ability of **HIV**-1-specific CTL clones of defined epitope specificity and HLA restriction to lyse infected CD4+ cells at serial time points following infection. CD4+ cell lines were acutely infected with **HIV**-1 IIIB at a high multiplicity of infection, and the kinetics of cell lysis were examined and compared with the kinetics of viral replication. Intracellular **HIV**-1 p24 expression was detected by 1 to 2 days after infection, reaching over 98% positive cells by day 4. Recognition of the infected cells by HLA A2- and B14-restricted CTL clones closely paralleled intracellular p24 expression and preceded peak virion production. The maximal levels of lysis with Gag-, reverse transcriptase-, and envelope-specific clones were different, however. The Gag- and envelope-specific clones lysed infected cells at levels equivalent to peptide-sensitized controls, whereas lysis by the reverse transcriptase-specific clones plateaued at a lower level. Peptide titration curves indicated that this effect was not due to differences in sensitivity to the cognate epitopes for the different clones. Although

...1 infection induced an approximately 50% decrease in class I MHC expression on the surface of infected cells, lysis by CTL clones was unaffected. These studies indicate that **HIV**-1-specific CTL can efficiently lyse **HIV**-1-infected CD4+ cells and suggest that the partial downregulation of class I molecules in infected cells does not significantly affect recognition by CTL.

L10 ANSWER 64 OF 101 MEDLINE on STN

96291770. PubMed ID: 8743084. Strong **cytotoxic** T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing **HIV** type 1 infection. Harrer T; Harrer E; Kalams S A; Elbeik T; Staprans S I; Feinberg M B; Cao Y; Ho D D; Yilma T; Caliendo A M; Johnson R P; Buchbinder S P; **Walker B D.** (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Boston, USA.) AIDS research and human retroviruses, (1996 May 1) 12 (7) 585-92. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Some individuals in well-defined cohorts have now been infected with **HIV**-1 for well over a decade and yet remain clinically asymptomatic with normal CD4 counts. To determine immunologic and virologic parameters in these individuals, we examined 10 persons from the San Francisco City Clinic with firmly documented infection of 11-15 years duration who had maintained stable CD4 counts above 500 cells/microliters. Our results indicate that long-term nonprogressors are a heterogeneous group with respect to viral load and **HIV**-1-specific immune responses, and that progression can occur even after 15 years of stable infection. However, in a subset of persons with the lowest viral loads and persistent nonprogressive infection, we detected strong CTL responses, whereas neutralizing antibody studies revealed weak to undetectable titers against a panel of 10 primary isolates. This study demonstrates that a vigorous in vivo activated **HIV**-1-specific CTL response can be part of the host immune response in stable nonprogressive **HIV**-1 infection, and that circulating activated CTL can be detected in the setting of an extremely low viral load. These results also indicate that long-term nonprogressing **HIV**-1 infection does not require the presence of broadly cross-reactive neutralizing antibodies.

L10 ANSWER 65 OF 101 MEDLINE on STN

96261668. PubMed ID: 8666925. T cell receptor usage and fine specificity of **human immunodeficiency virus** 1-specific **cytotoxic** T lymphocyte clones: analysis of quasispecies recognition reveals a dominant response directed against a minor in vivo variant. Kalams S A; Johnson R P; Dynan M J; Hartman K E; Harrer T; Harrer E; Trocha A K; Blattner W A; Buchbinder S P; **Walker B D.** (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Boston, 02114, USA.) Journal of experimental medicine, (1996 Apr 1) 183 (4) 1669-79. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Numerous virus-specific, class I-restricted **cytotoxic** T lymphocyte (CTL) epitopes have been identified, yet little information is available regarding the specificity of the CTL response in persons of the same human histocompatibility leukocyte antigen (HLA) type. In this study, the **human immunodeficiency virus** (**HIV**) 1 envelope-specific CTL response was evaluated in five HLA-B14-positive persons. CTL responses specific for a previously described nine-amino acid epitope in gp41 (aa 584-592, ERYLKDQQL) could be identified in all subjects, and CTL clones specific for this epitope could be isolated from four persons. Despite heterogeneous T cell receptor usage, the fine specificity of the clones was similar, as defined by recognition of alanine-substituted peptides as well as peptides representing natural **HIV**-1 sequence variants. Correlation with in vivo virus sequences revealed that the dominant species in two of the subjects represented poorly recognized variants, with a K-->Q substitution at amino acid 588, whereas no variants were observed in the other two subjects. Although clonal type-specific responses to these dominant variants could be identified, the magnitude of these responses remained small, and the dominant CTL response was directed at the minor in vivo variant. These studies indicate that despite similar

epitope specific immunologic pressure in persons of the same HLA type, the in vivo quasispecies may differ, and that the major in vivo immune response to a given CTL epitope can be directed at a minor variant.

L10 ANSWER 66 OF 101 MEDLINE on STN

96180222. PubMed ID: 8786327. **Cytotoxic** T lymphocytes in asymptomatic long-term nonprogressing **HIV**-1 infection. Breadth and specificity of the response and relation to in vivo viral quasispecies in a person with prolonged infection and low viral load. Harrer T; Harrer E; Kalams S A; Barbosa P; Trocha A; Johnson R P; Elbeik T; Feinberg M B; Buchbinder S P; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital and Harvard Mmedical School, Boston, MA 02114, USA.) Journal of immunology (Baltimore, Md. : 1950), (1996 Apr 1) 156 (7) 2616-23. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Although vigorous activated and memory CTL have been associated with **HIV**-1 infection, data are lacking regarding the breadth of epitopes recognized in a given individual and the relationship to the viral quasispecies present in vivo. In this study we performed a detailed analysis of the **HIV**-1-specific CTL response in a seropositive person with documented **HIV**-1 infection of 15 yr duration, stable CD4 counts above 500 cells/ml, and viral load persistently below 500 molecules of RNA/ml of plasma. Epitope mapping studies revealed the presence of HLA class I-restricted CTL responses to six different epitopes in p17, p24, RT, Env, and Nef, which conferred broadly cross-reactive recognition of reported **HIV**-1 variants. Sequence analysis of autologous viruses revealed the absence of immune escape variants within five of the six epitopes. Despite consistently low viral RNA levels in plasma and viral DNA levels in PBMC, in vivo-activated circulating CTL were detected against three of the epitopes. Five of the six epitopes, including the three dominant epitopes, have been detected in persons with progressive disease, suggesting that nonprogressors may not target unique epitopes. This study demonstrates that **HIV**-1-specific CTL can be highly activated and broadly directed in the setting of an extremely low viral load, and that neither high viral load nor antigenic diversity is required for the generation of a multispecific CTL response. Although the detection of strong CTL responses, low viral load, and lack of immune escape are consistent with the hypothesis that CTL may contribute to lack of disease progression in this individual, the contribution of these responses to maintenance of the asymptomatic state remains to be determined.

L10 ANSWER 67 OF 101 MEDLINE on STN

96162113. PubMed ID: 8568316. Recognition of the highly conserved YMDD region in the **human immunodeficiency virus** type 1 reverse transcriptase by HLA-A2-restricted **cytotoxic** T lymphocytes from an asymptomatic long-term nonprogressor. Harrer E; Harrer T; Barbosa P; Feinberg M; Johnson R P; Buchbinder S; **Walker B D**. (AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Boston 02114, USA.) Journal of infectious diseases, (1996 Feb) 173 (2) 476-9. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** (**HIV**) type 1 reverse transcriptase (RT) is an important target for therapeutic intervention and for **HIV**-1-specific **cytotoxic** T lymphocytes (CTL). An HLA-A2-restricted CTL epitope containing the sequence YMDD, which is highly conserved among human and animal **retroviruses** and essential for function of the RNA-dependent DNA polymerase, is identified. The drug resistance mutation at RT amino acid 184 (M184V), associated with (-)-2'-deoxy-3'-thiacytidine (lamivudine), (-)-2'-deoxy-5-fluoro-3'-thiacytidine (FTC), and dideoxyinosine resistance, is located within this epitope and abolishes recognition by an established CTL response. This study demonstrates that the CTL response may target functionally relevant regions of the RT protein and suggests drug therapy may select for viral variants with altered susceptibility to established cellular immune responses.

96074274. PubMed ID: 7583437. Inhibition of cytotoxicity and cytokine release of **CD8+ HIV**-specific **cytotoxic** T lymphocytes by pentoxifylline. Heinkelein M; Schneider-Schaulies J; **Walker B D**; Jassoy C. (Institute for Virology and Immunobiology, Wurzburg University, Germany.) Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association, (1995 Dec 1) 10 (4) 417-24. Journal code: 9501482. ISSN: 1077-9450. Pub. country: United States. Language: English.

AB **HIV**-specific **cytotoxic** T lymphocytes (CTLs) are an important component of the host immune response against **HIV** infection, and these cells release a variety of cytokines when they meet their target antigen. Since the phosphodiesterase inhibitor pentoxifylline is being used as a therapeutic agent in clinical trials of **HIV** infection due to its inhibitory effect on virus replication in vitro, we examined the effect of pentoxifylline on cytotoxicity and cytokine secretion by **HIV**-specific **CD8+** CTLs. Pentoxifylline inhibited cytotoxicity of CTLs and suppressed interferon-gamma, tumor necrosis factor-alpha, and granulocyte-macrophage colony-stimulating factor release by these cells at the transcription level. Suppression of cytokine release resulted in reduced capacity of the CTLs to induce HLA class I and ICAM-1 expression and to stimulate **HIV**-1 replication. These results suggest that inhibition of **HIV**-specific **CD8+** CTLs by pentoxifylline may be therapeutically relevant. Moreover, this study extends previous observations by demonstrating that, in addition to its ability to suppress cytokine production by macrophages and CD4+ T helper cells, pentoxifylline may inhibit cytotoxicity and cytokine secretion by antigen-specific **CD8+ cytotoxic** T lymphocytes.

L10 ANSWER 69 OF 101 MEDLINE on STN

96004229. PubMed ID: 7587372. **Cytotoxic** T lymphocytes and **HIV**-1 related neurologic disorders. Kalams S A; **Walker B D**. (Infectious Disease Unit, Harvard Medical School, Boston, MA, USA.) Current topics in microbiology and immunology, (1995) 202 79-88. Ref: 51. Journal code: 0110513. ISSN: 0070-217X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In summary, one can conclude that infected persons exhibit an extremely vigorous, virus-specific CTL response, and in at least some individuals this response is broadly directed at multiple epitopes. These cells are present at the time or initial control of viremia and can also be detected after more than a decade of asymptomatic infection. These cells can also be found in the central nervous system in persons with ADC, and one can envision pathways in which the inflammatory cytokines released by these cells upon activation could contribute to the neurologic sequelae of infection. However, the precise role of these cells as a protective host defense and the possible contribution of these cells, or products released by these cells, to tissue damage at sites such as the lung and brain remain to be determined. Further delineation of the role played by CTLs in the pathogenesis of disease should be extremely useful in helping to understand the disease itself and to guide intervention strategies.

L10 ANSWER 70 OF 101 MEDLINE on STN

95271010. PubMed ID: 7538543. An epitope-selective, transporter associated with antigen presentation (TAP)-1/2-independent pathway and a more general TAP-1/2-dependent antigen-processing pathway allow recognition of the **HIV**-1 envelope glycoprotein by **CD8+** CTL. Hammond S A; Johnson R P; Kalams S A; **Walker B D**; Takiguchi M; Safrit J T; Koup R A; Siliciano R F. (Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.) Journal of immunology (Baltimore, Md. : 1950), (1995 Jun 1) 154 (11) 6140-56. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The lysis of virally infected cells by CTLs requires the recognition of processed fragments of viral proteins presented in association with class I MHC molecules on the surfaces of infected cells. Processing begins in the cytosol with the degradation of viral proteins into peptides that are then transported into the endoplasmic reticulum (ER) for association with

newly synthesized class I molecules. Transport is mediated by a heterodimer of the MHC-encoded proteins, transporter associated with Ag presentation (TAP)-1 and TAP-2. Uncertainty exists over the site of processing of viral envelope (env) proteins. The extracellular domains of env proteins are not present in the cytosol, the site in which the class I-restricted Ag-processing pathway begins. Rather, the ecto-domains of env proteins are cotranslationally translocated into the ER during biosynthesis. We have analyzed the processing of the **HIV**-1 env protein by using a large series of env-specific human **CD8**+ CTL clones. These studies have led to the delineation of two distinct processing pathways. The first pathway permits a subset of class I-restricted epitopes in the ecto-domain of the env protein to be generated efficiently by a TAP-1/2-independent mechanism localized to the ER or a premedial Golgi compartment. A second, more general pathway that is capable of generating all env epitopes uses as a substrate env protein mislocalized to the cytosol and produces peptides that are transported from the cytoplasm to the ER in a TAP-1/2-dependent fashion.

L10 ANSWER 71 OF 101 MEDLINE on STN
95194735. PubMed ID: 7888228. **HIV** type 1-specific **cytotoxic** T lymphocytes stimulate HLA class I and intercellular adhesion molecule type 1 expression and increase beta 2-microglobulin levels in vitro. Jassoy C; Heinkelein M; Klinker H; **Walker B D**. (Institute for Virology and Immunobiology, University of Wurzburg, Germany.) AIDS research and human retroviruses, (1994 Dec) 10 (12) 1685-93. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Besides acting in a direct manner, cytolytic **HIV**-1-specific CTLs release a variety of cytokines. To assess the potential role of cytokines released by these CTLs we tested the ability of **soluble** products secreted by **HIV**-1-specific CTLs to induce HLA class I and ICAM-1 expression and to raise beta 2-microglobulin (beta 2M) concentrations in cell culture. To this end, supernatants were derived from **HIV**-1-specific CTLs incubated with autologous B lymphoblasts presenting either the cognate **HIV**-1 epitope or a control peptide. Cell lines and peripheral blood mononuclear cells (PBMCs) were incubated with these supernatants for 24-48 hr. Similarly, cells were cocultured with CTLs and their targets. This study demonstrates that in parallel with lysis of their cognate target, **HIV**-1-specific CTLs secreted products that stimulated HLA class I and ICAM-1 expression on cell lines and PBMCs. As few as 1000 CTLs significantly induced the expression of these molecules. In addition, secreted products of **HIV**-specific CTLs enhanced beta 2M release by PBMCs and Jurkat cells. These effects were mediated primarily by IFN-gamma and suggest that **HIV**-specific CTLs may contribute to increased HLA class I expression in infected tissue and elevated ICAM-1 and beta 2M concentrations in serum and cerebrospinal fluid of infected individuals.

L10 ANSWER 72 OF 101 MEDLINE on STN
95169520. PubMed ID: 7532426. **HIV**-1-specific **cytotoxic** T lymphocyte response in healthy, long-term nonprogressing seropositive persons. Harrer E; Harrer T; Buchbinder S; Mann D L; Feinberg M; Yilma T; Johnson R P; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston 02114.) AIDS research and human retroviruses, (1994) 10 Suppl 2 S77-8. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L10 ANSWER 73 OF 101 MEDLINE on STN
95169519. PubMed ID: 7532425. Epitope specificity of MHC restricted **cytotoxic** T lymphocytes induced by candidate **HIV**-1 vaccine. Johnson R P; Hammond S A; Trocha A; Siliciano R F; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Charlestown 02129.) AIDS research and human retroviruses, (1994) 10 Suppl 2 S73-5. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L10 ANSWER 74 OF 101 MEDLINE on STN
95169476. PubMed ID: 7865295. Conference on advances in AIDS vaccine

development 1993. Summary. Cytotoxic T cell immunity workshop. Walker B C; **Walker B D**; Mestecky J; Mathieson B J. (Vaccine Research and Development Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-7640.) AIDS research and human retroviruses, (1994) 10 Suppl 2 S177-9. Ref: 34. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L10 ANSWER 75 OF 101 MEDLINE on STN
95087232. PubMed ID: 7527747. **Cytotoxic CD8**+ T lymphocytes reactive with **human immunodeficiency virus**-1 produce granulocyte/macrophage colony-stimulating factor and variable amounts of interleukins 2, 3, and 4 following stimulation with the cognate epitope. Price P; Johnson R P; Scadden D T; Jassoy C; Rosenthal T; Kalams S; **Walker B D.** (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Clinical immunology and immunopathology, (1995 Jan) 74 (1) 100-6. Journal code: 0356637. ISSN: 0090-1229. Pub. country: United States. Language: English.
AB Infection with **human immunodeficiency virus** type 1 (**HIV**-1) induces vigorous and persistent **cytotoxic CD8**+ T cell responses. CTL clones were derived from peripheral blood or cerebrospinal fluid of three **HIV**-1 patients, with depressed CD4+ T cell counts. When stimulated with HLA-compatible target cells (B-LCL) presensitized with cognate **HIV**-1 peptides, all clones produced GM-CSF, TNF-alpha, and IFN-gamma and most produced low amounts of IL2, IL3, and IL4. After nonspecific stimulation with a phorbol ester and calcium ionophore, the clones secreted cytokines at levels similar to those from CD4+ lines from an **HIV**-1 infected donor. The ability of supernatants from the stimulated CTL clones to support the formation of granulocyte-macrophage colonies in normal bone marrow suggests that the GM-CSF was biologically active. Release of cytokines by activated CTL may influence the immunopathogenesis of **HIV** disease.

L10 ANSWER 76 OF 101 MEDLINE on STN
95016420. PubMed ID: 7523570. Naturally processed viral peptides recognized by **cytotoxic** T lymphocytes on cells chronically infected by **human immunodeficiency virus** type 1. Tsomides T J; Aldovini A; Johnson R P; **Walker B D**; Young R A; Eisen H N. (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139.) Journal of experimental medicine, (1994 Oct 1) 180 (4) 1283-93. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.
AB We have established long-term cultures of several cell lines stably and uniformly expressing **human immunodeficiency virus** type 1 (**HIV**-1) in order to (a) identify naturally processed **HIV**-1 peptides recognized by **cytotoxic** T lymphocytes (CTL) from **HIV**-1-seropositive individuals and (b) consider the hypothesis that naturally occurring epitope densities on **HIV**-infected cells may limit their lysis by CTL. Each of two A2-restricted **CD8**+ CTL specific for **HIV**-1 gag or reverse transcriptase (RT) recognized a single naturally processed **HIV**-1 peptide in trifluoroacetic acid (TFA) extracts of infected cells: gag 77-85 (SLYNTVATL) or RT 476-484 (ILKEPVHGV). Both processed peptides match the synthetic peptides that are optimally active in cytotoxicity assays and have the consensus motif described for A2-associated peptides. Their abundances were approximately 400 and approximately 12 molecules per infected Jurkat-A2 cell, respectively. Other synthetic **HIV**-1 peptides active at subnanomolar concentrations were not present in infected cells. Except for the antigen processing mutant line T2, **HIV**-infected HLA-A2+ cell lines were specifically lysed by both A2-restricted CTL, although infected Jurkat-A2 cells were lysed more poorly by RT-specific CTL than by gag-specific CTL, suggesting that low cell surface density of a natural peptide may limit the effectiveness of some **HIV**-specific CTL despite their vigorous activity against synthetic peptide-treated target cells.

L10 ANSWER 77 OF 101 MEDLINE on STN
95008926. PubMed ID: 7523033. **Cytotoxic** T lymphocytes in **human immunodeficiency virus** infection: responses to structural proteins. Johnson R P; **Walker B D.** (Infectious Disease Unit, Massachusetts General

microbiology and immunology, (1994) 189 35-63. Ref: 120. Journal code: 0110513. ISSN: 0070-217X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L10 ANSWER 78 OF 101 MEDLINE on STN

95008666. PubMed ID: 7924192. The **cytotoxic** T-lymphocyte response in **HIV**-1 infection. Kalams S A; **Walker B D.** (Massachusetts General Hospital Infectious Disease Unit, Boston.) Clinics in laboratory medicine, (1994 Jun) 14 (2) 271-99. Ref: 146. Journal code: 8100174. ISSN: 0272-2712. Pub. country: United States. Language: English.

AB **HIV** infection is associated with an extremely vigorous virus-specific **cytotoxic** T-lymphocyte (CTL) response. This CTL activity is of sufficient magnitude to be detected using freshly isolated peripheral blood mononuclear cells, but despite this vigorous immune response, **HIV**-1 disease ultimately progresses. This article describes methods used to detect CTL responses and epitopes recognized by **HIV**-1 specific CTL. The potential role of CTL in the control of viral replication, disease pathogenesis, and possible mechanisms that allow **HIV**-1 to ultimately evade the host's immune response is discussed. Finally, efforts to induce CTL responses through vaccines are summarized.

L10 ANSWER 79 OF 101 MEDLINE on STN

94238428. PubMed ID: 8182510. The rationale for immunotherapy in **HIV**-1 infection. **Walker B D.** (Harvard Medical School, Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of acquired immune deficiency syndromes, (1994) 7 Suppl 1 S6-13. Ref: 53. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) infection causes progressive and ultimately profound immunosuppression. Initially, however, infection is associated with vigorous virus-specific immune responses, including both neutralizing antibodies and **cytotoxic** T lymphocytes (CTLs). Although the host immune response is ultimately unable to eliminate the virus, experimental data suggest that these immune responses help to inhibit virus replication during the prolonged asymptomatic phase of illness. A number of mechanisms have been proposed to contribute to viral persistence in infected persons, among them direct immunosuppressive effects of the virus; defects in antigen presentation; down-modulation of human leukocyte antigens (HLA); clonal deletion of existing immune responses; sequence variation leading to immune escape; and decreased T-helper cell function. The rationale supporting the use of vaccine therapy in **HIV**-1 infection is based on the hypothesis that viral persistence is due to an inadequate immune response generated by natural infection and that the immune system can be induced to generate more effective immunoregulatory responses by vaccination. Potential mechanisms by which this might occur include enhanced clearance of circulating virus, enhanced recognition of virus variants, enhanced presentation of viral antigens to the immune system, and increased regional T-cell help. A number of protocols evaluating vaccine therapy in **HIV**-1 infection are presently under way, the results of which should facilitate rational decisions regarding the use of this approach in **HIV**-1-infected persons.

L10 ANSWER 80 OF 101 MEDLINE on STN

94202302. PubMed ID: 7908700. Induction of a major histocompatibility complex class I-restricted **cytotoxic** T-lymphocyte response to a highly conserved region of **human immunodeficiency virus** type 1 (**HIV**-1) gp120 in seronegative humans immunized with a candidate **HIV**-1 vaccine. Johnson R P; Hammond S A; Trocha A; Siliciano R F; **Walker B D.** (Infectious Disease Unit, Massachusetts General Hospital, Charlestown 02129.) Journal of virology, (1994 May) 68 (5) 3145-53. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Efforts to induce broadly reactive immunity against **human immunodeficiency virus** type 1 (**HIV**-1) have been impaired by the extent of sequence variation exhibited by this **lentivirus**. **Cytotoxic** T lymphocytes (CTL) specific for other viruses such as influenza virus have been shown to mediate immunity against divergent viral strains, a

properly and is related to the ability of CTL to recognize processed antigen derived from conserved viral proteins. A recent candidate **HIV**-1 vaccine regimen has been described in which subjects receive a primary immunization with a recombinant vaccinia virus expressing gp160 and then a booster immunization with recombinant gp160. Volunteers immunized with this regimen have exhibited augmented humoral responses and have also developed CD4+ and **CD8**+ CTL specific for gp160. In this report, we have identified the epitopes recognized by CD4+ and **CD8**+ CTL obtained from two vaccines. An immunodominant **CD8**+ CTL response was HLA-A3.1 restricted and recognized a 10-amino-acid epitope (gp120/38-47) in a highly conserved region of gp120. CTL specific for the epitope gp120/38-47 were able to lyse targets sensitized with peptides corresponding to all known natural sequence variants in this region. In addition, other HLA class I-restricted CTL epitopes were identified in relatively conserved regions of gp120 and gp41, and CD4+ CTL were shown to recognize two different regions of gp120. Thus, in these two volunteers, immunization with a single strain of **HIV**-1 induced CD4+ and **CD8**+ CTL that are specific for multiple conserved regions of **HIV**-1 and would be expected to recognize a broad range of viral isolates.

L10 ANSWER 81 OF 101 MEDLINE on STN

94194282. PubMed ID: 8145043. Longitudinal analysis of T cell receptor (TCR) gene usage by **human immunodeficiency virus** 1 envelope-specific **cytotoxic** T lymphocyte clones reveals a limited TCR repertoire. Kalams S A; Johnson R P; Trocha A K; Dynan M J; Ngo H S; D'Aquila R T; Kurnick J T; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Journal of experimental medicine, (1994 Apr 1) 179 (4) 1261-71. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** 1 (**HIV**-1) infection is associated with a vigorous cellular immune response that allows detection of **cytotoxic** T lymphocyte (CTL) activity using freshly isolated peripheral blood mononuclear cells (PBMC). Although restricting class I antigens and epitopes recognized by **HIV**-1-specific CTL have been defined, the effector cells mediating this vigorous response have been characterized less well. Specifically, no studies have addressed the breadth and duration of response to a defined epitope. In the present study, a longitudinal analysis of T cell receptor (TCR) gene usage by CTL clones was performed in a seropositive person using TCR gene sequences as a means of tracking responses to a well-defined epitope in the glycoprotein 41 transmembrane protein. 10 CTL clones specific for this human histocompatibility leukocyte antigen-B14-restricted epitope were isolated at multiple time points over a 31-mo period. All clones were derived from a single asymptomatic **HIV**-1-infected individual with a vigorous response to this epitope that was detectable using unstimulated PBMC. Polymerase chain reaction amplification using V alpha and V beta family-specific primers was performed on each clone, followed by DNA sequencing of the V-D-J regions. All 10 clones utilized V alpha 14 and V beta 4 genes. Sequence analysis of the TCR revealed the first nine clones isolated to also be identical at the nucleotide level. The TCR-alpha junctional region sequence of the tenth clone was identical to the junctional region sequences of the other nine, but this clone utilized distinct D beta and J beta gene segments. This study provides evidence that the observed high degree of **HIV**-1-specific CTL activity may be due to monoclonal or oligoclonal expansion of specific effector cells, and that progeny of a particular CTL clone may persist for prolonged periods in vivo in the presence of a chronic productive viral infection. The observed limited TCR diversity against an immunodominant epitope may limit recognition of virus variants with mutations in regions interacting with the TCR, thereby facilitating immune escape.

L10 ANSWER 82 OF 101 MEDLINE on STN

93390998. PubMed ID: 1285013. Viruses, chemotherapy and immunity. Koziel M J; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Parasitology, (1992) 105 Suppl S85-92. Ref: 63. Journal code: 0401121. ISSN: 0031-1820. Pub. country: ENGLAND: United Kingdom. Language:

English.

AB An increasing number of **antiviral** agents are presently in various stages of development and testing, and an increasing number have recently been licensed for use in humans. These drugs have been used extensively to treat viral infections in immunocompromised individuals, and these studies indicate that for many **antiviral** agents the response to therapy is highly dependent on the integrity of the underlying host immune response. In particular, the response to zidovudine, acyclovir and ganciclovir in persons with **HIV**-1 infection is highly dependent upon CD4 number, which can be considered a surrogate marker for the state of host immune function in these subjects. Responses to interferons likewise can be shown to depend on the host immune response, with responses due to both direct **antiviral** effects of this agent as well as immunomodulatory effects mediated through interferon-induced upregulation of HLA molecule expression. The interdependence of host immunity with **antiviral** efficacy is underscored by the increased **antiviral** drug resistance in persons with advanced degrees of chronic immunosuppression, related to the higher level of viral replication and viraemia which occurs in the absence of an effective host immune response. Further definition of the precise mechanisms of these interactions should facilitate the rational design of **antiviral** agents and immunomodulatory therapies to improve treatment of viral infections.

L10 ANSWER 83 OF 101 MEDLINE on STN

93371734. PubMed ID: 7689848. **Cytotoxic** T-cell epitopes in **HIV/SIV** infection. Venet A; **Walker B D.** (Laboratoire d'Immunologie et d'Oncologie des Maladies Retrovirales, Unite INSERM U152, Institut Cochin de Genetique Moleculaire, Paris, France.) AIDS (London, England), (1993) 7 Suppl 1 S117-26. Ref: 107. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

L10 ANSWER 84 OF 101 MEDLINE on STN

93301829. PubMed ID: 8315573. Induction of **HIV**-1 replication in a chronically infected T-cell line by **cytotoxic** T lymphocytes. Harrer T; Jassoy C; Harrer E; Johnson R P; **Walker B D.** (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of acquired immune deficiency syndromes, (1993 Aug) 6 (8) 865-71. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB **CD8**-positive **cytotoxic** T cells (CTLs) are activated by recognition of peptide bound to MHC class I molecules on target cells. This human leukocyte antigen-restricted process induces not only lysis of target cells but also secretion of lymphokines by the CTLs, including TNF-alpha, TNF-beta, and IFN-gamma. In this study we show that activation of **HIV**-1-specific CTL clones by their cognate peptide epitopes induces **HIV**-1 replication in the chronically **HIV**-1-infected T-cell line ACH-2. The **HIV**-1-inducing activity correlates with increased levels of TNF-alpha produced by these CTLs, and can be inhibited by anti-TNF-alpha antibodies, indicating that the effect is mediated by this cytokine. These studies suggest that activation of CTL in vivo could lead to enhanced viral replication. Although **HIV**-1-specific CTLs may serve as a host defense to inhibit virus replication, the induction of TNF-alpha production by these cells may facilitate viral replication in infected bystander cells, contributing to viral persistence and disease pathogenesis.

L10 ANSWER 85 OF 101 MEDLINE on STN

93271830. PubMed ID: 1726962. Identification of **HIV**-1 **cytotoxic** T-lymphocyte epitopes and implications for vaccine development. Johnson R P; **Walker B D.** (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Biotechnology therapeutics, (1991) 2 (1-2) 137-46. Ref: 31. Journal code: 8918082. ISSN: 0898-2848. Pub. country: United States. Language: English.

L10 ANSWER 86 OF 101 MEDLINE on STN

93233253. PubMed ID: 7682629. **Human immunodeficiency virus** type 1-specific **cytotoxic** T lymphocytes release gamma interferon, tumor

target antigens. Jassoy C; Harrer T; Rosenthal T; Navia B A; Worth J; Johnson R P; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Journal of virology, (1993 May) 67 (5) 2844-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) infection is associated with elevated levels of inflammatory cytokines in the serum and cerebrospinal fluid of infected persons, but the sources of these proteins as well as the specific stimuli which trigger their production and release have not been fully defined. In this study, we evaluated the ability of **HIV**-1-specific **cytotoxic** T-lymphocyte (CTL) clones derived from seropositive persons to release gamma interferon (IFN-gamma), tumor necrosis factor alpha (TNF-alpha), and TNF-beta upon contact with target cells presenting viral antigen. Peripheral blood- and cerebrospinal fluid-derived **HIV**-1-specific CD3+ CD4- **CD8**+ CTL clones as well as freshly isolated peripheral blood mononuclear cells from infected persons were tested in parallel for **HIV**-1-specific cytotoxicity and cytokine release. Target cells consisted of autologous and allogeneic B-lymphoblastoid cell lines sensitized with synthetic **HIV**-1 peptides containing the epitopes recognized by these CTL. Cytokine production was measured by specific enzyme-linked immunosorbent assay of culture supernatant fluid. **HIV**-1-specific CTL clones directed at envelope, Gag, reverse transcriptase, and Nef epitopes specifically released IFN-gamma, TNF-alpha, and TNF-beta upon contact with their relevant target epitopes but not following contact with irrelevant epitopes. These cytokines were released in an HLA class I-restricted fashion, and release was detectable as early as 4 to 6 h of incubation and remained elevated at 48 h. Fresh peripheral blood mononuclear cells from a seropositive person likewise released IFN-gamma in an antigen-specific and HLA class I-restricted manner when incubated with target cells presenting a peptide containing a CTL epitope, paralleling the **HIV**-specific cytolytic activity of these cells. These studies indicate that in addition to mediating direct cytotoxicity, **HIV**-1-specific CTL may affect other immune responses by releasing IFN-gamma, TNF-alpha, and TNF-beta. Elevated levels of these cytokines which have been detected in serum and cerebrospinal fluid of infected persons may be due at least in part to the persistent **HIV**-1-specific CTL response.

L10 ANSWER 87 OF 101 MEDLINE on STN

93171601. PubMed ID: 7679694. Presentation of endogenous peptides to MHC class I-restricted **cytotoxic** T lymphocytes in transport deletion mutant T2 cells. Zweerink H J; Gammon M C; Utz U; Sauma S Y; Harrer T; Hawkins J C; Johnson R P; Sirotina A; Hermes J D; **Walker B D**; +. (Department of Autoimmune Disease Research, Merck Research Laboratories, Rahway, NJ 07065.) Journal of immunology (Baltimore, Md. : 1950), (1993 Mar 1) 150 (5) 1763-71. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The ability of minigene-encoded viral peptide epitopes to be presented by class I molecules in the absence of MHC-encoded transporters has been evaluated in mutant T2 cells. These cells have a large deletion in the class II MHC region that includes the known transporter protein for antigenic peptides and proteasome genes and they are defective in presenting viral epitopes to CTL. T2 cells that express minigenes encoding the influenza virus matrix peptide 58-66 (GILGFVFTL) and two HTLV 1 Tax peptides 11-19 (LLFGYPVYV) and 12-19 were lysed by HLA-A2-restricted peptide-specific CTL. Minigene expression of a HLA-A2-restricted **HIV** reverse transcriptase peptide 476-484 (ILKEPVHGV) with three charged residues sensitized T2 cells poorly for lysis by **HIV**-specific CTL unless the peptide was preceded by an endoplasmic reticulum translocation signal sequence. Expression of an influenza virus nucleoprotein peptide 383-391 (SRYWAIRTR) with three charged arginine residues did sensitize HLA-B27+ T2 cells for lysis by peptide-specific CTL. These and other results with endogenously expressed peptide analogs in which hydrophobic and charged amino acids were interchanged demonstrate that antigenic peptides can be translocated from the cytoplasm into the class I Ag presentation pathway

independent of the encoded transporters, and side peptide hydrophobicity appears not to be a major determinant in selecting peptides for this alternate pathway.

L10 ANSWER 88 OF 101 MEDLINE on STN

93152287. PubMed ID: 7678973. CD4+ **human immunodeficiency virus** type 1 (**HIV**-1) envelope-specific **cytotoxic** T lymphocytes derived from the peripheral blood cells of an **HIV**-1-infected individual. Curiel T J; Wong J T; Gorczyca P F; Schooley R T; **Walker B D.** (Department of Medicine, University of Colorado Health Sciences Center, Denver 80262.) AIDS research and human retroviruses, (1993 Jan) 9 (1) 61-8. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Virus-specific **cytotoxic** T lymphocytes (CTL) are frequently of the **CD8**+ surface phenotype, although CTL of the CD4+ surface phenotype have also been described. Published reports of CTL derived from peripheral blood mononuclear cells (PBMC) of individuals infected with **human immunodeficiency virus** type 1 (**HIV**-1) have described primarily cells of the **CD8**+ surface phenotype. However, CD4+ **HIV**-1 envelope-specific CTL have been reported after in vitro stimulation with **HIV**-1 envelope protein of peripheral blood cells obtained from **HIV**-1-seronegative donors, in peripheral blood cells after vaccination of **HIV**-1-seronegative persons with **HIV**-1 envelope proteins, and in cerebrospinal fluid cells of **HIV**-1-infected individuals. Recently, CD4+ **HIV**-1 gag-specific CTL were also reported. We now report a patient from whom we derived **HIV**-1 envelope-specific CTL cell lines of the CD4+ surface phenotype. Our cell culture technique did not employ exogenous viral antigenic stimulation, and may therefore yield cells that more closely reflect those in the underlying populations from which they were derived. These CTL did not appear to have the clear human leukocyte antigen (HLA) class II restriction pattern typically seen in CD4-expressing cells and were not functionally inhibited by anti-CD3 antibody. Further work will be required to define the role of CD4+ CTL in the pathogenesis of **HIV**-1 disease.

L10 ANSWER 89 OF 101 MEDLINE on STN

93100827. PubMed ID: 7677956. Recognition of a highly conserved region of **human immunodeficiency virus** type 1 gp120 by an HLA-Cw4-restricted **cytotoxic** T-lymphocyte clone. Johnson R P; Trocha A; Buchanan T M; **Walker B D.** (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of virology, (1993 Jan) 67 (1) 438-45. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) isolates exhibit extensive sequence variation, particularly in the gp120 subunit of the envelope glycoprotein, and the degree of this variation has raised questions as to whether conserved regions of the **HIV**-1 envelope can be recognized by the host immune response. A **CD8**+ **cytotoxic** T-lymphocyte (CTL) clone specific for the **HIV**-1 envelope was derived by culturing peripheral blood mononuclear cells from an **HIV**-1 seropositive subject in the presence of a CD3-specific monoclonal antibody, interleukin-2, and irradiated allogeneic peripheral blood mononuclear cells. Lysis of target cells was restricted by an HLA-C molecule, Cw4, which has not been previously shown to present viral antigen to CTL. Mapping of the specificity of this CTL clone by using synthetic **HIV**-1 peptides localized the epitope to an 8-amino-acid region of gp120 (amino acids 376 to 383) which is conserved among approximately 90% of sequenced viral isolates. Examination of the recognition of variant peptides by this CTL clone demonstrated that a single, nonconservative amino acid substitution within the 8-amino-acid minimal epitope could abrogate lysis of targets incubated with the variant peptide. The identification of a CTL epitope in a highly conserved region of gp120 documents the ability of cellular immune responses of infected persons to respond to relatively invariant portions of this highly variable envelope glycoprotein. However, the ability of even a single-amino-acid change in gp120 to abolish lysis by CTL supports the hypothesis that sequence variation in **HIV**-1 may serve as a mechanism of immune escape. In addition, the identification of an

for these molecules.

L10 ANSWER 90 OF 101 MEDLINE on STN

93017933. PubMed ID: 1383338. Detection of a vigorous **HIV**-1-specific **cytotoxic** T lymphocyte response in cerebrospinal fluid from infected persons with AIDS dementia complex. Jassoy C; Johnson R P; Navia B A; Worth J; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of immunology (Baltimore, Md. : 1950), (1992 Nov 1) 149 (9) 3113-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB AIDS dementia complex is a common neurologic disorder in later stages of **HIV**-1 infection. Because virus-specific CTL have been shown to contribute to neurologic disease in certain viral illnesses, we examined the cerebrospinal fluid of **HIV**-1-infected persons with various stages of AIDS dementia complex for the presence of **HIV**-1-specific CTL. In five of six subjects studied, **HIV**-1-specific CTL were identified in the cerebrospinal fluid. These CTL were directed at epitopes within the gag, reverse transcriptase, envelope, and nef proteins and restricted by HLA class I Ag. In four of these subjects, virus-specific CTL were detected in higher numbers in the cerebrospinal fluid compared to the peripheral blood, suggesting a specific recruitment to or local induction within the nervous system. These studies demonstrate the presence of a vigorous and broadly directed CTL response to **HIV**-1 in the central nervous system of infected persons with AIDS dementia complex, and provide immunologic evidence of localized intrathecal infection. Although **HIV**-1-specific CTL may serve to inhibit viral replication in the central nervous system, the presence of a persistent CTL response in the central nervous system may also contribute to the neurologic disorders characteristic of **HIV**-1 infection.

L10 ANSWER 91 OF 101 MEDLINE on STN

92202878. PubMed ID: 1372650. Identification of overlapping HLA class I-restricted **cytotoxic** T cell epitopes in a conserved region of the **human immunodeficiency virus** type 1 envelope glycoprotein: definition of minimum epitopes and analysis of the effects of sequence variation. Johnson R P; Trocha A; Buchanan T M; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of experimental medicine, (1992 Apr 1) 175 (4) 961-71. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Although the immunologic basis of protective immunity in **human immunodeficiency virus** type 1 (**HIV**-1) infection has not yet been defined, virus-specific **cytotoxic** T lymphocytes (CTL) are likely to be an important host defense and may be a critical feature of an effective vaccine. These observations, along with the inclusion of the **HIV**-1 envelope in the majority of vaccine candidates presently in clinical trials, underscore the importance of the precise characterization of the cellular immune responses to this protein. Although humoral immune responses to the envelope protein have been extensively characterized, relatively little information is available regarding the envelope epitopes recognized by virus-specific CTL and the effects of sequence variation within these epitopes. Here we report the identification of two overlapping CTL epitopes in a highly conserved region of the **HIV**-1 transmembrane envelope protein, gp41, using CTL clones derived from two seropositive subjects. An eight-amino acid peptide was defined as the minimum epitope recognized by HLA-B8-restricted CTL derived from one subject, and in a second subject, an overlapping nine-amino acid peptide was identified as the minimal epitope for HLA-B14-restricted CTL clones. Selected single amino acid substitutions representing those found in naturally occurring **HIV**-1 isolates resulted in partial to complete loss of recognition of these epitopes. These data indicate the presence of a highly conserved region in the **HIV**-1 envelope glycoprotein that is immunogenic for CTL responses. In addition, they suggest that natural sequence variation may lead to escape from immune detection by **HIV**-1-specific CTL. Since the region containing these epitopes has been previously shown to contain an immunodominant B cell epitope and also

overlaps with a major histocompatibility complex class II T cell epitope recognized by CD4+ CTL from **HIV**-1 rgp160 vaccine recipients, it may be particularly important for **HIV**-1 vaccine development. Finally, the identification of minimal CTL epitopes presented by class I HLA molecules should facilitate the definition of allele-specific motifs.

L10 ANSWER 92 OF 101 MEDLINE on STN

92107932. PubMed ID: 1722325. An optimal viral peptide recognized by **CD8**+ T cells binds very tightly to the restricting class I major histocompatibility complex protein on intact cells but not to the purified class I protein. Tsomides T J; **Walker B D**; Eisen H N. (Department of Biology, Massachusetts Institute of Technology, Cambridge 02139.) Proceedings of the National Academy of Sciences of the United States of America, (1991 Dec 15) 88 (24) 11276-80. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB **CD8**+ **cytotoxic** T lymphocytes recognize cell surface complexes formed by class I major histocompatibility complex (MHC-I) glycoproteins and antigenic peptides. We have identified a peptide nonamer (termed IV9) derived from the **human immunodeficiency virus** that is over a millionfold more active (at subpicomolar concentrations) than peptide analogues longer or shorter by one or two amino acid residues. Although IV9 does not detectably bind to isolated MHC-I molecules as measured by equilibrium dialysis, we quantitated its specific binding in unaltered form to MHC-I on intact cells. Less than 1% of cell surface MHC-I forms complexes with IV9, which suffices to trigger maximal **cytotoxic** T-lymphocyte activity. By contrast, a peptide dodecamer that includes the IV9 sequence and is active at micromolar concentrations does not bind to MHC-I on intact cells, raising the possibility that this longer peptide undergoes processing. Using stoichiometrically iodinated IV9 to obviate the ambiguities associated with trace labeling methods, we measured the dissociation kinetics of purified peptide/MHC-I complexes isolated by affinity chromatography and found these complexes to be exceedingly stable (t1/2 = 200-600 hr).

L10 ANSWER 93 OF 101 MEDLINE on STN

91349569. PubMed ID: 1715361. **HIV**-1 gag-specific **cytotoxic** T lymphocytes recognize multiple highly conserved epitopes. Fine specificity of the gag-specific response defined by using unstimulated peripheral blood mononuclear cells and cloned effector cells. Johnson R P; Trocha A; Yang L; Mazzara G P; Panicali D L; Buchanan T M; **Walker B D**. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of immunology (Baltimore, Md. : 1950), (1991 Sep 1) 147 (5) 1512-21. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB CTL directed at the highly conserved **HIV**-1 gag protein have been described in **HIV**-1 seropositive persons and may be an important host defense against this **retrovirus**. Presently only limited data are available regarding the specific epitopes recognized by these CTL. In this study, we have performed a detailed examination of the gag-specific CTL response in three **HIV**-1 seropositive subjects, using both unstimulated PBMC and cloned CTL. Lysis of gag-expressing targets was found to be mediated by CD3+**CD8**+ lymphocytes and restricted by class I Ag. Multiple class I Ag were found to restrict gag epitopes in each subject studied, with as many as three of these Ag involved in presenting gag CTL epitopes in a single subject. The majority of gag-specific CTL activity was found to be directed against epitopes in the p24 subunit of the gag protein, with at least seven different HLA class I-restricted CTL p24 epitopes identified in these three subjects. Less CTL activity was directed against p17 subunit of gag and two CTL epitopes were identified in this protein. Although as many as four different epitopes in gag were recognized using CTL from a single subject, none of the epitopes was recognized by CTL from more than one subject. Analysis of gag epitope recognition using cloned CTL demonstrated heterogeneity and specificity not appreciated using unstimulated PBMC. The identification of multiple relatively conserved epitopes in the **HIV**-1 gag protein and the heterogeneity of CTL responses to this protein may have important

implications for vaccine development and our understanding of AIDS pathogenesis.

L10 ANSWER 94 OF 101 MEDLINE on STN
91073289. PubMed ID: 1845771. **HIV**-1 infection does not induce tumor necrosis factor-alpha or interferon-beta gene transcription. Goldfeld A E; Birch-Limberger K; Schooley R T; **Walker B D**. (Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts.) Journal of acquired immune deficiency syndromes, (1991) 4 (1) 41-7. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB An early host defense against infection by RNA or DNA viruses is the induction, within infected cells, of tumor necrosis factor-alpha (TNF-alpha) gene transcription. The protein product of the TNF-alpha gene alone, or together with different types of interferons, inhibits viral propagation in diverse cell types. In this study, the effect of acute and chronic **human immunodeficiency virus** type 1 (**HIV**-1) infection on the transcription of the TNF-alpha and interferon-beta (IFN-beta) genes was examined in susceptible monocyte and T-cell lines as well as in primary human mononuclear phagocytes. Although Sendai virus, a prototypic inducer of TNF-alpha and IFN-beta mRNA, induced the transcription of both genes in the monocyte cell lines and TNF-alpha in the T-cell line and in primary mononuclear phagocytes, transcription of these genes was not inducible by **HIV**-1. Therefore, **HIV**-1 was able to infect these cells without triggering the transcription of genes encoding proteins important in immediate **antiviral** cellular defenses. These results may explain in part how **HIV**-1 is able to establish persistent intracellular infections and escape acute host responses that have evolved to combat viral infection.

L10 ANSWER 95 OF 101 MEDLINE on STN
90274890. PubMed ID: 1693514. **Cytotoxic** T lymphocytes against **HIV**. **Walker B D**; Plata F. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) AIDS (London, England), (1990 Mar) 4 (3) 177-84. Ref: 65. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB **HIV**-1 infection has clearly been shown to induce a vigorous CTL response in infected people, and this response is present at a time when immune function otherwise is globally impaired. **HIV**-1-specific CTL are detectable both in peripheral blood and tissues of infected people, and are aimed at multiple viral proteins. The precise epitopes recognized by these CTL are now being defined, and the establishment of CTL clones should facilitate further functional analysis of these cells. However, the central question as to the clinical relevance of **HIV**-1-specific CTL remains. By analogy with animal model systems of virus infection, it is reasonable to postulate that **HIV**-1-specific CTL serve a protective role as a host defense. In this regard, in vitro data indicate that **HIV**-1-specific CTL can suppress viral replication, and longitudinal clinical studies indicate that the vigorous CTL activity seen in the early stages of infection declines with disease progression. Alternatively, the presence of **HIV**-1-specific CTL in tissues such as the lung and brain have to at least raise the possibility that these cells may be contributing to the pathologic consequences of infection. In addition, the relative protective effects of virus-specific CTL compared to other effector mechanisms such as ADCC and neutralizing antibodies remain to be determined. Nevertheless, recent data in the **SIV** vaccine model give reason for encouragement that a state of protective immunity can be achieved in AIDS-like illness caused by **retroviruses**. The search continues presently not only for the parameters which define protective immunity in **HIV**-1 infection, but also for the ideal **HIV**-1 immunogens to be used for vaccination of human populations.

L10 ANSWER 96 OF 101 MEDLINE on STN
90083298. PubMed ID: 2480604. Long-term culture and fine specificity of human **cytotoxic** T-lymphocyte clones reactive with **human immunodeficiency virus** type 1. **Walker B D**; Flexner C;

Schooley R T. (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Proceedings of the National Academy of Sciences of the United States of America, (1989 Dec) 86 (23) 9514-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The definition of **human immunodeficiency virus** type 1 (**HIV**-1) immunogenic epitopes is central to the rational design of AIDS vaccine strategies. In this study, we have generated seven **HIV**-1 reverse transcriptase-specific **cytotoxic** T-lymphocyte (CTL) clones from the peripheral blood of two seropositive subjects. Epitopes recognized by these CTL clones were identified by using target cells infected with recombinant **HIV**-1-vaccinia virus vectors expressing truncated reverse transcriptase proteins and further defined by using target cells incubated with overlapping 25-amino acid synthetic reverse transcriptase peptides. Five different CTL epitopes were identified, and in each case recognition was restricted by class I human leukocyte antigens (HLA). Clones maintained specific cytolytic function in continuous culture for up to 11 months, requiring only periodic restimulation with a CD3-specific monoclonal antibody. These results indicate that **HIV**-1-specific, major histocompatibility class I-restricted CTL recognize multiple epitopes of a single viral gene product in conjunction with different host HLA antigens. In addition, they demonstrate that human virus-specific CTL can be grown in long-term culture without the need for reexposure to viral antigen.

L10 ANSWER 97 OF 101 MEDLINE on STN

89215353. PubMed ID: 2785146. Synergistic inhibition of **human immunodeficiency virus** type 1 (**HIV**-1) replication in vitro by recombinant **soluble** CD4 and 3'-azido-3'-deoxythymidine. Johnson V A; Barlow M A; Chou T C; Fisher R A; **Walker B D**; Hirsch M S; Schooley R T. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114.) Journal of infectious diseases, (1989 May) 159 (5) 837-44. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB A combination of **antiviral** therapies that target different sites in the **human immunodeficiency virus** type 1 (**HIV**-1) replicative cycle may be necessary for optimal treatment of **HIV**-1 infections. We evaluated the interactions of a **soluble** virus receptor (recombinant **soluble** CD4 or rsT4) and a reverse transcriptase inhibitor (azidothymidine, AZT) against **HIV**-1 replication in vitro. A variety of cell types was studied including peripheral blood mononuclear cells, a CD4-positive T-cell line, and a CD4-positive human monocyte cell line. The combination of rsT4 and AZT inhibited **HIV**-1 synergistically over a broad range of drug concentrations and multiplicities of infection in several different **HIV**-1 replication assays. Drug interactions were evaluated by the median-effect principle and the isobologram technique using a computer analysis. In all of the cell types tested, combinations of rsT4 and AZT were synergistic in vitro, without additive cytotoxicity.

L10 ANSWER 98 OF 101 MEDLINE on STN

88178071. PubMed ID: 2451288. **HIV**-1 reverse transcriptase is a target for **cytotoxic** T lymphocytes in infected individuals. **Walker B D**; Flexner C; Paradis T J; Fuller T C; Hirsch M S; Schooley R T; Moss B. (Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.) Science, (1988 Apr 1) 240 (4848) 64-6. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Characterization of the host immune response to **human immunodeficiency virus** type 1 (**HIV**-1) is critical to the rational design of an effective AIDS vaccine. In this study, **cytotoxic** T lymphocytes (CTL) specific for **HIV**-1 reverse transcriptase (RNA-dependent DNA polymerase) were found in blood samples from **HIV**-1-infected individuals. CTL targets were prepared by immortalizing B cells from ten seropositive and six seronegative individuals, and then infecting these cells with recombinant vaccinia viruses containing **HIV**-1 genes. CTL directed against autologous B lymphoblasts expressing **HIV**-1 reverse transcriptase were detected in fresh blood samples from eight **HIV**-1 seropositive subjects, but in no seronegative controls. The effector cells were

lymphocytes. Because the **HIV**-1 pol gene is highly conserved among different isolates and generates both humoral and cellular immune responses, it bears consideration for inclusion in a candidate AIDS vaccine.

L10 ANSWER 99 OF 101 MEDLINE on STN

88122540. PubMed ID: 2829022. **HIV** infection is blocked in vitro by recombinant **soluble** CD4. Fisher R A; Bertonis J M; Meier W; Johnson V A; Costopoulos D S; Liu T; Tizard R; **Walker B D**; Hirsch M S; Schooley R T; +. (Biogen Research Corporation, Cambridge, Massachusetts 02142.) Nature, (1988 Jan 7) 331 (6151) 76-8. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The T-cell surface glycoprotein, CD4 (T4), acts as the cellular receptor for **human immunodeficiency virus**, type 1 (**HIV**-1), the first member of the family of viruses that cause acquired immunodeficiency syndrome. **HIV** recognition of CD4 is probably mediated through the virus envelope glycoprotein (gp120) as shown by co-immunoprecipitation of CD4 and gp120 (ref.5) and by experiments using recombinant gp120 as a binding probe. Here we demonstrate that recombinant **soluble** CD4(rsT4) purified from the conditioned medium of a stably transfected Chinese hamster ovary cell line is a potent inhibitor of both virus replication and virus-induced cell fusion (syncytium formation). These results suggest that rsT4 is sufficient to bind **HIV**, and that it represents a potential anti-viral therapy for **HIV** infection.

L10 ANSWER 100 OF 101 MEDLINE on STN

88068547. PubMed ID: 2825177. Inhibition of **human immunodeficiency virus** syncytium formation and virus replication by castanospermine. **Walker B D**; Kowalski M; Goh W C; Kozarsky K; Krieger M; Rosen C; Rohrschneider L; Haseltine W A; Sodroski J. (Infectious Disease Unit, Massachusetts General Hospital, Boston.) Proceedings of the National Academy of Sciences of the United States of America, (1987 Nov) 84 (22) 8120-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine) is a plant alkaloid that modifies glycosylation by inhibiting alpha-glucosidase I. Castanospermine is shown to inhibit syncytium formation induced by the envelope glycoprotein of the **human immunodeficiency virus** and to inhibit viral replication. The decrease in syncytium formation in the presence of castanospermine can be attributed to inhibition of processing of the envelope precursor protein gp160, with resultant decreased cell surface expression of the mature envelope glycoprotein gp120. In addition, castanospermine may cause defects in steps involved in membrane fusion after binding of CD4 antigen. The **antiviral** effects of castanospermine may be due to modifications of the envelope glycoprotein that affect the ability of the virus to enter cells after attachment to the CD4 cell receptor.

L10 ANSWER 101 OF 101 MEDLINE on STN

87258273. PubMed ID: 3496541. **HIV**-specific **cytotoxic** T lymphocytes in seropositive individuals. **Walker B D**; Chakrabarti S; Moss B; Paradis T J; Flynn T; Durno A G; Blumberg R S; Kaplan J C; Hirsch M S; Schooley R T. Nature, (1987 Jul 23-29) 328 (6128) 345-8. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Virus-specific **cytotoxic** T lymphocytes (CTL) which kill virus-infected cells are thought to be a major host defence against viral infections. Here we report the existence of **human immunodeficiency virus** (**HIV**)-specific CTL in persons infected with this virus, the aetiological agent of AIDS (acquired immunodeficiency syndrome). Recombinant **HIV**-vaccinia viruses were used to express **HIV** antigens in B-cell lines established from subjects seropositive for **HIV** and seronegative controls. Circulating lymphocytes capable of killing **HIV** env-expressing autologous B cells were detected in eight of eight seropositive subjects; in addition, at least three seropositive subjects demonstrated gag-specific **cytotoxic** responses. No **HIV**-specific cytotoxicity was

observed in seronegative subjects. Selective inhibition of the env-specific cytotoxicity by a CD3-specific monoclonal antibody indicates that the effectors are T cells. This demonstration of a **cytotoxic** T-cell immune response to **HIV** in infected individuals should prove useful in investigating the immunopathogenesis of **HIV** infection further and in evaluating AIDS vaccine strategies.

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(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
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L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
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L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
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E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
E WALKER B D/AU
L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

=> file uspatful

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	30.71	88.27

FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 29 Apr 2004 (20040429/PD)
FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2004

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

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>>>  USPATFULL and USPAT2 can be accessed and searched together        <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

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E3            0 --> GEIBEN-LYNN R/IN
E4            2     GEIBERGER AXEL/IN
E5            6     GEIBERGER CHRISTOPH/IN
E6            1     GEIBIG KARL F/IN
E7            1     GEIBIG ROLF/IN
E8            4     GEIBL MATTHIAS/IN
E9            2     GEIBLE HARRY F/IN
E10           2     GEIBLE NORMAN P/IN
E11           1     GEIBLER MICHAEL/IN
E12           1     GEICK ANKE/IN

=> s e2
L11           1 "GEIBEN LYNN RALF"/IN

=> d l11,cbib,ab

L11  ANSWER 1 OF 1  USPATFULL on STN
2002:235509 Serpin drugs for treatment of HIV infection and method of use
    thereof.
    Geiben Lynn, Ralf, Watertown, MA, UNITED STATES
    Walker, Bruce D., Milton, MA, UNITED STATES
    US 2002127698 A1 20020912
    APPLICATION: US 2002-57613  A1  20020125 (10)
    PRIORITY: US 2001-264338P  20010126 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention includes compositions comprising substantially purified
       serpin that are useful in methods for the treatment and prevention of
       HIV infection. The invention also includes methods for the treatment and
       prevention of HIV infection comprising contacting a composition of the
       invention with a human patient or treating HIV infection by introducing
       into a cell susceptible to HIV infection a DNA molecule encoding a
       serpin. Additionally, the invention includes antibodies and kits useful
       in the detection, treatment, and prevention of HIV infection.


=> s (peroxiredoxin? or NKEF? or natural killer enhancing factor? or PRX?)
            59 PEROXIREDOXIN?
            40 NKEF?
        471525 NATURAL
          9476 KILLER
        183479 ENHANCING
        793115 FACTOR?
            21 NATURAL KILLER ENHANCING FACTOR?
                 (NATURAL(W)KILLER(W)ENHANCING(W)FACTOR?)
           437 PRX?
L12        526 (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
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=> s l12 and (HIV or human immunodeficiency virus or SIV or simian immunodeficiency virus or le
         30564 HIV
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          17663 IMMUNODEFICIENCY
          72758 VIRUS
          12633 HUMAN IMMUNODEFICIENCY VIRUS
                  (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
           2039 SIV
          12170 SIMIAN
          17663 IMMUNODEFICIENCY
          72758 VIRUS
           1119 SIMIAN IMMUNODEFICIENCY VIRUS
                  (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
           3825 LENTIVIR?
          30171 RETROVIR?
L13         120 L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
                IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR RETROVIR?)

=> s l13 and (treat? or therapeut? or antivir?)
         953381 TREAT?
         162331 THERAPEUT?
          20022 ANTIVIR?
L14         120 L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)

=> s l14 and (peroxiredox?/clm or NKEF?/clm or natural killer enhancing/clm)
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              6 NKEF?/CLM
          46888 NATURAL/CLM
            606 KILLER/CLM
          25732 ENHANCING/CLM
              1 NATURAL KILLER ENHANCING/CLM
                  ((NATURAL(W)KILLER(W)ENHANCING)/CLM)
L15          11 L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHANCI
               NG/CLM)

=> d his

     (FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

     FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
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                E WALKER BRUCE D/IN
L2            7 S E3
L3            6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
                E LYNN R G/IN
L4            2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
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                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4
                E WALKER B D/AU
L6          155 S E3
L7          154 S L6 NOT L5
L8            0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9          127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10         101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
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L12 [illegible]
L13 120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14 120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15 11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA

=> s l15 not (l1 or l11)
L16 10 L15 NOT (L1 OR L11)

=> d l16,cbib,1-10

L16 ANSWER 1 OF 10 USPATFULL on STN
2004:70050 Strong gene sets for glioma classification.
Zhang, Wei, Houston, TX, UNITED STATES
Fuller, Greg, Houston, TX, UNITED STATES
Dougherty, Ed, College Station, TX, UNITED STATES
Hess, Kenneth, Houston, TX, UNITED STATES
Board of Regents, The University of Texas System (U.S. corporation)The Texas A&M University System (U.S. corporation)
US 2004053277 A1 20040318
APPLICATION: US 2003-390343 A1 20030317 (10)
PRIORITY: US 2002-364608P 20020315 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 2 OF 10 USPATFULL on STN
2004:63761 Method for detecting methylation states for a toxicological diagnostic.
Olek, Alexander, Berlin, GERMANY, FEDERAL REPUBLIC OF
Piepenbrock, Christian, Berlin, GERMANY, FEDERAL REPUBLIC OF
Berlin, Kurt, Stahnsdorf, GERMANY, FEDERAL REPUBLIC OF
US 2004048279 A1 20040311
APPLICATION: US 2003-416905 A1 20030514 (10)
WO 2001-EP12951 20011108
PRIORITY: DE 2000-10056802 20001114
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 3 OF 10 USPATFULL on STN
2003:324320 Inhibition of tumor growth via **peroxiredoxin** 3.
Dang, Chi V., Baltimore, MD, UNITED STATES
Wonsey, Diane, Concord, MA, UNITED STATES
The John Hopkins University, Baltimore, MD (U.S. corporation)
US 2003228294 A1 20031211
APPLICATION: US 2003-408766 A1 20030408 (10)
PRIORITY: US 2002-370873P 20020408 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 4 OF 10 USPATFULL on STN
2003:283120 Regeneration of endogenous myocardial tissue by induction of neovascularization.
Itescu, Silviu, New York, NY, UNITED STATES
US 2003199464 A1 20031023
APPLICATION: US 2002-128738 A1 20020423 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 5 OF 10 USPATFULL on STN
2003:265822 Medicinal compositions.
Awaya, Akira, Kanagawa, JAPAN
Onodera, Takashi, Ibaraki, JAPAN
Kakegawa, Tomohito, Chiba, JAPAN
US 2003186839 A1 20031002
APPLICATION: US 2003-362218 A1 20030226 (10)
WO 2001-JP7354 20010828
PRIORITY: JP 2000-256943 20000828

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 6 OF 10 USPATFULL on STN
2003:239335 SCA2 knockout animal and methods of use.
Pulst, Stefan M., Los Angeles, CA, UNITED STATES
US 2003167495 A1 20030904
APPLICATION: US 2002-141541 A1 20020507 (10)
PRIORITY: US 2001-289231P 20010507 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 7 OF 10 USPATFULL on STN
2002:272437 Compositions comprising mixtures of **therapeutic** proteins and methods of producing the same.
Lau, Allan S., Pok Fu Lam, HONG KONG
Wan, Winnie H., Woodside, CA, UNITED STATES
Browning, Laura, Brentwood, CA, UNITED STATES
Ossina, Natalya, Albany, CA, UNITED STATES
US 2002150552 A1 20021017
APPLICATION: US 2001-952843 A1 20010911 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 8 OF 10 USPATFULL on STN
2002:198230 Natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, UNITED STATES
Yu, Guo-Liang, Darnestown, MD, UNITED STATES
Gentz, Reiner, Silver Spring, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
US 2002106323 A1 20020808
APPLICATION: US 2001-911346 A1 20010724 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 9 OF 10 USPATFULL on STN
2001:102589 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6255079 B1 20010703
APPLICATION: US 1995-467265 19950606 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L16 ANSWER 10 OF 10 USPATFULL on STN
1999:146312 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, 305 W. Side Dr., Apt. #204, Gaithersburg, MD, United States 20878
Yu, Guo-Liang, 13524 Straw Bale La., Darnestown, MD, United States 20878
Gentz, Reiner, 13404 Fairland Park Dr., Silver Spring, MD, United States 20904
Rosen, Craig A., 22400 Rolling Hill Rd., Laytonsville, MD, United States 20882
US 5985612 19991116
APPLICATION: US 1995-467265 19950606 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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L16 ANSWER 8 OF 10 USPATFULL on STN

Ni, Jian, Gaithersburg, MD, UNITED STATES
Yu, Guo-Liang, Darnestown, MD, UNITED STATES
Gentz, Reiner, Silver Spring, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
US 2002106323 A1 20020808
APPLICATION: US 2001-911346 A1 20010724 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A human natural killer cell enhancing factor C and fragments thereof and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptidees by recombinant techniques is disclosed. Further disclosed are antibodies directed against such polypeptides and fragments or portions thereof and methods for producing such antibodies and utilizing such antibodies for **therapeutic** or diagnostic purposes. Also disclosed are methods for utilizing such polypeptides and/or antibodies for preventing and/or **treating** viral infections, inflammation, neoplasia and damge from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

CLM What is claimed is:

1. An isolated antibody or portion thereof that specifically binds to a protein whose sequence consists of amino acid residues +31 to +271 of SEQ ID NO:2.

2. The antibody or portion thereof of claim 1 wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

3. The antibody or portion thereof of claim 1 which is a monoclonal antibody.

4. The antibody or portion thereof of claim 1 which is a polyclonal antibody.

5. The antibody or portion thereof of claim 1 which is a chimeric antibody.

6. The antibody or portion thereof of claim 1 which is a single chain antibody.

7. The antibody or portion thereof of claim 1 which is a Fab fragment.

8. The antibody or portion thereof of claim 1 which is labeled.

9. The antibody of claim 8 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

10. A composition comprising the antibody or portion thereof of claim 1 and a carrier.

11. The composition of claim 10, wherein the antibody or portion thereof is a monoclonal antibody.

12. The composition of claim 10, wherein the antibody or portion thereof is a polyclonal antibody.

13. The composition of claim 10, wherein the antibody or portion thereof is a chimeric antibody.

14. The composition of claim 10, wherein the antibody or portion thereof is a single chain antibody.

15. The composition of claim 10, wherein the antibody or portion thereof

is a Fab fragment.

16. The composition of claim 10, wherein the antibody or portion thereof is labeled.

17. The composition of claim 16 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

18. An isolated cell that produces the antibody or portion thereof of claim 1.

19. A hybridoma that produces the antibody of claim 1.

20. A hybridoma that produces the antibody of claim 3.

21. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 1; and (b) detecting the **NKEF** C protein in the biological sample.

22. The method of claim 21 wherein the antibody is a monoclonal antibody.

23. The method of claim 21 wherein the antibody is a polyclonal antibody.

24. The method of claim 21 wherein the antibody is a chimeric antibody.

25. The method of claim 21 wherein the antibody is a single chain antibody.

26. The method of claim 21 wherein the antibody is a Fab fragment.

27. The method of claim 21 wherein the antibody is a labeled antibody.

28. The method of claim 27 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

29. An isolated antibody or portion thereof produced by immunizing an animal with a protein whose sequence comprises amino acid residues +31 to +271 of SEQ ID NO:2; wherein said antibody or portion thereof specifically binds to the amino acid sequence of SEQ ID NO:2.

30. An isolated antibody or portion thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose sequence consists of amino acid residues +1 to +271 of SEQ ID NO:2; (b) a protein whose sequence consists of at least 30 contiguous amino acid residues of SEQ ID NO:2; and (c) a protein whose sequence consists of at least 50 contiguous amino acid residues of SEQ ID NO:2.

31. The isolated antibody or portion thereof of claim 30, that specifically binds protein (a).

32. The isolated antibody or portion thereof of claim 30, that specifically binds protein (b).

33. The isolated antibody or portion thereof of claim 30, that specifically binds protein (c).

34. The isolated antibody or portion thereof of claim 30, wherein said protein specifically bound by said isolated antibody or portion thereof is glycosylated.

35. The isolated antibody or portion thereof of claim 30 which is a

monoclonal antibody.

36. The isolated antibody or portion thereof of claim 30 which is a polyclonal antibody.

37. The isolated antibody or portion thereof of claim 30, which is a chimeric antibody.

38. The isolated antibody or portion thereof of claim 30 which is a single chain antibody.

39. The isolated antibody or portion thereof of claim 30 which is a Fab fragment.

40. The antibody or portion thereof of claim 30 which is labeled.

41. The antibody of claim 40 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

42. A composition comprising the isolated antibody or portion thereof of claim 30 and a carrier.

43. The composition of claim 42, wherein the isolated antibody or portion thereof is a monoclonal antibody.

44. The composition of claim 42, wherein the isolated antibody or portion thereof is a polyclonal antibody.

45. The composition of claim 42, wherein the isolated antibody or portion thereof is a chimeric antibody.

46. The composition of claim 42, wherein the isolated antibody or portion thereof is a single chain antibody.

47. The composition of claim 42, wherein the isolated antibody or portion thereof is a Fab fragment.

48. The composition of claim 42, wherein the antibody or portion thereof is labeled.

49. The composition of claim 48 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

50. An isolated cell that produces the antibody of claim 30.

51. A hybridoma that produces the antibody of claim 30.

52. A hybridoma that produces the antibody of claim 35.

53. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the isolated antibody or portion thereof of claim 30; and (b) detecting **NKEF** C protein in the biological sample.

54. The method of claim 53 wherein the isolated antibody or portion thereof is a monoclonal antibody.

55. The method of claim 53 wherein the isolated antibody or portion thereof is a polyclonal antibody.

56. The method of claim 53 wherein the isolated antibody or portion thereof is a chimeric antibody.

57. The method of claim 53 wherein the isolated antibody or portion

wherein the antibody is a single chain antibody.

58. The method of claim 53 wherein the antibody is a Fab fragment.

59. The method of claim 53 wherein the antibody is a labeled antibody.

60. The method of claim 59 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

61. An antibody or portion thereof produced by immunizing an animal with a protein selected from the group consisting of: (a) a protein whose sequence comprises amino acid residues +1 to +271 of SEQ ID NO:2; (b) a protein whose sequence comprises 30 contiguous amino acid residues of SEQ ID NO:2; and (c) a protein whose sequence comprises 50 contiguous amino acid residues of SEQ ID NO:2; wherein said antibody or portion thereof specifically binds to the amino acid sequence of SEQ ID NO:2.

62. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (a).

63. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (b).

64. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (c).

65. An isolated antibody or portion thereof that specifically binds to a protein whose sequence consists of the amino acid sequence of the mature form of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

66. The antibody or portion thereof of claim 65 wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

67. The antibody or portion thereof of claim 65 which is a monoclonal antibody.

68. The antibody or portion thereof of claim 65 which is a polyclonal antibody.

69. The antibody or portion thereof of claim 65 which is a chimeric antibody.

70. The antibody or portion thereof of claim 65 which is a single chain antibody.

71. The antibody or portion thereof of claim 65 which is a Fab fragment.

72. The antibody or portion thereof of claim 65 which is labeled.

73. The antibody of claim 72 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

74. A composition comprising the antibody or portion thereof of claim 65 and a carrier.

75. The composition of claim 74, wherein the antibody or portion thereof is a monoclonal antibody.

76. The composition of claim 74, wherein the antibody or portion thereof is a chimeric antibody.

77. The composition of claim 74, wherein the antibody or portion thereof is a single chain antibody.

78. The composition of claim 74, wherein the antibody or portion thereof is a Fab fragment.

79. The composition of claim 74, wherein the antibody or portion thereof is labeled.

80. The composition of claim 79 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

81. An isolated cell that produces the antibody of claim 65.

82. A hybridoma that produces the antibody of claim 65.

83. A hybridoma that produces the antibody of claim 67.

84. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 65; and (b) detecting the **NKEF** C protein in the biological sample.

85. The method of claim 84 wherein the antibody is a monoclonal antibody.

86. The method of claim 84 wherein the antibody is a polyclonal antibody.

87. The method of claim 84 wherein the antibody is a chimeric antibody.

88. The method of claim 84 wherein the antibody is a single chain antibody.

89. The method of claim 84 wherein the antibody is a Fab fragment.

90. The method of claim 84 wherein the antibody is a labeled antibody.

91. The method of claim 90 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

92. An isolated antibody or portion thereof produced by immunizing an animal with a protein whose sequence comprises the amino acid sequence of the mature form of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; wherein said antibody or portion thereof specifically binds to the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97103.

93. An isolated antibody or portion thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose sequence consists of the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; (b) a protein whose sequence consists of 30 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; and (c) a protein whose sequence consists of 50 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

94. The isolated antibody or portion thereof of claim 93 that specifically binds protein (a).

95. The isolated antibody or portion thereof of claim 93 that specifically binds protein (b).

96. The isolated antibody or portion thereof of claim 93 that specifically binds protein (c).

97. The isolated antibody or portion thereof of claim 93, wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

98. The isolated antibody or portion thereof of claim 93, which is a monoclonal antibody.

99. The isolated antibody or portion thereof of claim 93, which is a polyclonal antibody.

100. The isolated antibody or portion thereof of claim 93, which is a chimeric antibody.

101. The isolated antibody or portion thereof of claim 93 which is a single chain antibody.

102. The isolated antibody or portion thereof of claim 93 which is a Fab fragment.

103. The isolated antibody or portion thereof of claim 93 which is labeled.

104. The isolated antibody or portion thereof of claim 103 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

105. A composition comprising the isolated antibody or portion thereof of claim 93 and a carrier.

106. The composition of claim 105, wherein the antibody or portion thereof is a monoclonal antibody.

107. The composition of claim 105, wherein the antibody or portion thereof is a polyclonal antibody.

108. The composition of claim 105, wherein the antibody or portion thereof is a chimeric antibody.

109. The composition of claim 105, wherein the antibody or portion thereof is a single chain antibody.

110. The composition of claim 105, wherein the antibody or portion thereof is a Fab fragment.

111. The composition of claim 105, wherein the antibody or portion thereof is labeled.

112. The composition of claim 111, wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

113. An isolated cell that produces the isolated antibody or portion thereof of claim 93.

114. A hybridoma that produces the antibody of claim 93.

115. A hybridoma that produces the antibody of claim 98.

116. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample from a test subject with the isolated antibody or portion thereof of claim 93; and (b) detecting **NKEF** C protein in the biological sample.

117. The method of claim 116, wherein the antibody or portion thereof is a monoclonal antibody.

118. The method of claim 116, wherein the antibody or portion thereof is a polyclonal antibody.

119. The method of claim 116, wherein the antibody or portion thereof is a chimeric antibody.

120. The method of claim 116, wherein the antibody or portion thereof is a single chain antibody.

121. The method of claim 116, wherein the antibody or portion thereof is a Fab fragment.

122. The method of claim 116, wherein the antibody or portion thereof is labeled.

123. The method of claim 122, wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

124. An antibody or portion thereof produced by immunizing an animal with a protein selected from the group consisting of: (a) a protein whose sequence comprises the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; (b) a protein whose sequence comprises at least 30 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; and (c) a protein whose sequence comprises at least 50 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; wherein said antibody or portion thereof specifically binds to the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

125. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (a).

126. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (b).

127. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (c).

128. A method of **treating** a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 1.

129. The method of claim 128, wherein the antibody is a monoclonal antibody.

130. A method of **treating** a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 30.

131. A method of **treating** a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 65.

132. A method of **treating** a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 93.

L16 ANSWER 9 OF 10 USPATFULL on STN
2001:102589 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States

Rubin, Craig M., Gaithersburg, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6255079 B1 20010703
APPLICATION: US 1995-467265 19950606 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or **treating** viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

CLM What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

2. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (a).

3. The polynucleotide of claim 2, which comprises nucleotides 31 to 843 of SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (b).

5. The polynucleotide of claim 4, which comprises nucleotides 34 to 843 of SEQ ID NO:1.

6. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (c).

7. The polynucleotide of claim 6, which comprises nucleotides 121-843.

8. The isolated nucleic acid molecule of claim 1 fused to a heterologous polynucleotide.

9. The isolated nucleic acid molecule of claim 8, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

10. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is DNA.

11. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is double stranded.

12. A recombinant vector comprising the nucleic acid molecule of claim

13. A recombinant host comprising the nucleic acid molecule of claim 1 operatively associated with a heterologous regulatory sequence.

14. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 13 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

15. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (d).

16. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (e).

17. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (f).

18. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (g).

19. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (h).

20. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (i).

21. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (j).

22. An isolated nucleic acid molecule consisting of a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

23. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (a).

24. The polynucleotide of claim 23, which comprises nucleotides 31 to 843 of SEQ ID NO:1.

25. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (b).

26. The polynucleotide of claim 25, which comprises nucleotides 34 to 843 of SEQ ID NO:1.

27. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (c).

28. The polynucleotide of claim 27, which comprises nucleotides 121-843.

29. The isolated nucleic acid molecule of claim 22 fused to a heterologous polynucleotide.

30. The isolated nucleic acid molecule of claim 29, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

31. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is DNA.

32. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is double stranded.

33. A recombinant vector comprising the nucleic acid molecule of claim 22.

34. A recombinant host comprising the nucleic acid molecule of claim 22 operatively associated with a heterologous regulatory sequence.

35. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 34 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

36. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (d).

37. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (e).

38. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (f).

39. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (g).

40. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (h).

41. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (i).

42. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (j).

L16 ANSWER 10 OF 10 USPATFULL on STN
1999:146312 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, 305 W. Side Dr., Apt. #204, Gaithersburg, MD, United States 20878
Yu, Guo-Liang, 13524 Straw Bale La., Darnestown, MD, United States 20878
Gentz, Reiner, 13404 Fairland Park Dr., Silver Spring, MD, United States 20904
Rosen, Craig A., 22400 Rolling Hill Rd., Laytonsville, MD, United States 20882
US 5985612 19991116
APPLICATION: US 1995-467265 19950606 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or **treating** viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting

diseases, for example, cancer, are also disclosed.

CLM What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

2. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (a).

3. The polynucleotide of claim 2, which comprises nucleotides 31 to 843 of SEQ ID NO: 1.

4. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (b).

5. The polynucleotide of claim 4, which comprises nucleotides 34 to 843 of SEQ ID NO: 1.

6. The isolated nucleic acid molecule of claim 3, wherein said polynucleotide is (c).

7. The polynucleotide of claim 6, which comprises nucleotides 121-843.

8. The isolated nucleic acid molecule of claim 1 fused to a heterologous polynucleotide.

9. The isolated nucleic acid molecule of claim 8, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

10. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is DNA.

11. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is double stranded.

12. A recombinant vector comprising the nucleic acid molecule of claim 1.

13. A recombinant host comprising the nucleic acid molecule of claim 1 operatively associated with a heterologous regulatory sequence.

14. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 13 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

15. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (d).

16. The isolated nucleic acid molecule of claim 1, wherein said

polynucleotide is (e).

17. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (f).

18. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (g).

19. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (h).

20. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (i).

21. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (j).

22. An isolated nucleic acid molecule consisting of a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

23. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (a).

24. The polynucleotide of claim 23, which comprises nucleotides 31 to 843 of SEQ ID NO: 1.

25. The isolated nucleic acid molecule of claim 2, wherein said polynucleotide is (b).

26. The polynucleotide of claim 25, which comprises nucleotides 34 to 843.of SEQ ID NO: 1.

27. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (c).

28. The polynucleotide of claim 25, which comprises nucleotides 121-843.

29. The isolated nucleic acid molecule of claim 22 fused to a heterologous polynucleotide.

30. The isolated nucleic acid molecule of claim 29, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

31. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is DNA.

32. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is double stranded.

33. A recombinant vector comprising the nucleic acid molecule of claim

claim 22.

34. A recombinant host comprising the nucleic acid molecule of claim 22 operatively associated with a heterologous regulatory sequence.

35. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 34 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

36. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (d).

37. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (e).

38. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (f).

39. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (g).

40. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (h).

41. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (i).

42. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (j).

=> d his

(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
E LYNN RALF GEIBEN/IN
L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
E LYNN R G/IN
L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
E LYNN R G/AU
E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
E WALKER B D/AU
L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
E GEIBEN-LYNN R/IN
L11 1 S E2

```
L13          120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14          120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15           11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16           10 S L15 NOT (L1 OR L11)

=> s l13 not l15
L17         109 L13 NOT L15

=> s l17 and ay<2002
        3448540 AY<2002
L18          54 L17 AND AY<2002

=> d l18,ti,1-54

L18  ANSWER 1 OF 54  USPATFULL on STN
TI     Diagnostic microarray for inflammatory bowel disease, crohn's disease
       and ulcerative colitis

L18  ANSWER 2 OF 54  USPATFULL on STN
TI     DNA array sequence selection

L18  ANSWER 3 OF 54  USPATFULL on STN
TI     Novel nucleic acids and polypeptides

L18  ANSWER 4 OF 54  USPATFULL on STN
TI     Modified nuclear glucocorticoid receptor, fusion protein, and DNA
       fragments coding for said receptor and said fusion protein

L18  ANSWER 5 OF 54  USPATFULL on STN
TI     Transgenic animal model by mineralocorticoid receptor antisense
       expression

L18  ANSWER 6 OF 54  USPATFULL on STN
TI     Gene expression in bladder tumors

L18  ANSWER 7 OF 54  USPATFULL on STN
TI     Transriotion factors

L18  ANSWER 8 OF 54  USPATFULL on STN
TI     Method for modifying plant morphology, biochemistry and physiology

L18  ANSWER 9 OF 54  USPATFULL on STN
TI     Methods for producing libraries of expressible gene sequences

L18  ANSWER 10 OF 54  USPATFULL on STN
TI     Seleno-cysteine containing protein zsel1

L18  ANSWER 11 OF 54  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of ovarian cancer

L18  ANSWER 12 OF 54  USPATFULL on STN
TI     Ovarian tumor sequences and methods of use therefor

L18  ANSWER 13 OF 54  USPATFULL on STN
TI     Methods of determining SAM-dependent methyltransferase activity using a
       mutant SAH hydrolase

L18  ANSWER 14 OF 54  USPATFULL on STN
TI     Methods and compositions for diagnosing and treating rheumatoid
       arthritis

L18  ANSWER 15 OF 54  USPATFULL on STN
TI     Gene expression profiling of primary breast carcinomas using arrays of
       candidate genes
```

L18 ANSWER 16 OF 54 USPATFULL on STN
TI Seleno-cysteine containing protein zsnk13

L18 ANSWER 17 OF 54 USPATFULL on STN
TI Nucleic acids, proteins, and antibodies

L18 ANSWER 18 OF 54 USPATFULL on STN
TI Libraries of expressible gene sequences

L18 ANSWER 19 OF 54 USPATFULL on STN
TI Gene and sequence variation associated with lipid disorder

L18 ANSWER 20 OF 54 USPATFULL on STN
TI Gene and sequence variation associated with cancer

L18 ANSWER 21 OF 54 USPATFULL on STN
TI Polynucleotides encoding or regulating electron transfer molecules

L18 ANSWER 22 OF 54 USPATFULL on STN
TI Combined growth factor-deleted and thymidine kinase-deleted vaccinia virus vector

L18 ANSWER 23 OF 54 USPATFULL on STN
TI Prohormone convertase transformed cells and polypeptide synthesis

L18 ANSWER 24 OF 54 USPATFULL on STN
TI RGS compositions and therapeutic and diagnostic uses therefor

L18 ANSWER 25 OF 54 USPATFULL on STN
TI 33167, a novel human hydrolase and uses therefor

L18 ANSWER 26 OF 54 USPATFULL on STN
TI Compositions and methods for the therapy and diagnosis of ovarian cancer

L18 ANSWER 27 OF 54 USPATFULL on STN
TI Polynucleotides and polypeptides derived from corn ear

L18 ANSWER 28 OF 54 USPATFULL on STN
TI Stress-regulated genes of plants, transgenic plants containing same, and methods of use

L18 ANSWER 29 OF 54 USPATFULL on STN
TI Antioxidant protein 2, gene and methods of use therefor

L18 ANSWER 30 OF 54 USPATFULL on STN
TI Recombinant animal viral nucleic acids

L18 ANSWER 31 OF 54 USPATFULL on STN
TI Inducible expression system

L18 ANSWER 32 OF 54 USPATFULL on STN
TI Method for reducing angiogenesis by administration of a scatter factor inhibitor

L18 ANSWER 33 OF 54 USPATFULL on STN
TI Selective amplification of target polynucleotide sequences

L18 ANSWER 34 OF 54 USPATFULL on STN
TI Peptides blocking vascular endothelial growth factor (VEGF) -mediated angiogenesis, polynucleotides encoding said peptides and methods of use thereof

L18 ANSWER 35 OF 54 USPATFULL on STN
TI Methods and compositions for inhibiting thrombin generation at the surface of cells

L18 ANSWER 36 OF 54 USPATFULL on STN
TI Taxol resistance associated gene

L18 ANSWER 37 OF 54 USPATFULL on STN
TI Human stress array

L18 ANSWER 38 OF 54 USPATFULL on STN
TI Compositions and methods for the therapy and diagnosis of ovarian cancer

L18 ANSWER 39 OF 54 USPATFULL on STN
TI Methods and compositions for the diagnosis and treatment of cataracts

L18 ANSWER 40 OF 54 USPATFULL on STN
TI Natural killer cell enhancing factor C

L18 ANSWER 41 OF 54 USPATFULL on STN
TI Recombinant animal viral nucleic acids

L18 ANSWER 42 OF 54 USPATFULL on STN
TI Acridone-derived compounds useful as antineoplastic and antiretroviral agents

L18 ANSWER 43 OF 54 USPATFULL on STN
TI Method for preparing a recombinant adenovirus genome

L18 ANSWER 44 OF 54 USPATFULL on STN
TI Acridone-derived compounds useful as antineoplastic and antiretroviral agents

L18 ANSWER 45 OF 54 USPATFULL on STN
TI Selective amplification of target polynucleotide sequences

L18 ANSWER 46 OF 54 USPATFULL on STN
TI Recombinant animal viral nucleic acids

L18 ANSWER 47 OF 54 USPATFULL on STN
TI Methods of assaying differential expression

L18 ANSWER 48 OF 54 USPATFULL on STN
TI Recombinant plant viral nucleic acids

L18 ANSWER 49 OF 54 USPATFULL on STN
TI Recombinant plant viral nucleic acids

L18 ANSWER 50 OF 54 USPATFULL on STN
TI Plasmid for production of CRM protein and diphtheria toxin

L18 ANSWER 51 OF 54 USPATFULL on STN
TI DNA's encoding natural killer cell enhancing factor

L18 ANSWER 52 OF 54 USPATFULL on STN
TI Recombinant plant viral nucleic acids

L18 ANSWER 53 OF 54 USPATFULL on STN
TI Method for processing blood for human transfusion

L18 ANSWER 54 OF 54 USPATFULL on STN
TI Plant viral vectors having heterologous subgenomic promoters for systemic expression of foreign genes

=> d his

(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

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     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
                E LYNN RALF GEIBEN/IN
L1            1 S E3
                E WALKER BRUCE D/IN
L2            7 S E3
L3            6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
                E LYNN R G/IN
L4            2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
                E LYNN R G/AU
                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4
                E WALKER B D/AU
L6          155 S E3
L7          154 S L6 NOT L5
L8            0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9          127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10         101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
L12         526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13         120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14         120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15          11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16          10 S L15 NOT (L1 OR L11)
L17         109 S L13 NOT L15
L18          54 S L17 AND AY<2002

=> file medline
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               35.52     123.79

FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004

 FILE LAST UPDATED: 1 MAY 2004 (20040501/UP).  FILE COVERS 1951 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details. OLDMEDLINE now back to 1951.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s (peroxiredox? or NKEF? or natural killer enhancing factor? or Prx?)
          336 PEROXIREDOX?
           19 NKEF?
       178186 NATURAL
        32159 KILLER
        34354 ENHANCING
      2122472 FACTOR?
           22 NATURAL KILLER ENHANCING FACTOR?
                (NATURAL(W)KILLER(W)ENHANCING(W)FACTOR?)
          290 PRX?
```

PRX?)

=> s l19 and (HIV or human immunodeficiency virus)
136286 HIV
8502943 HUMAN
112869 IMMUNODEFICIENCY
373812 VIRUS
42705 HUMAN IMMUNODEFICIENCY VIRUS
(HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L20 3 L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> d l20,cbib,ab,1-3

L20 ANSWER 1 OF 3 MEDLINE on STN
2003032310. PubMed ID: 12421812. **HIV**-1 antiviral activity of recombinant natural killer cell enhancing factors, **NKEF**-A and **NKEF**-B, members of the **peroxiredoxin** family. Geiben-Lynn Ralf; Kursar Mischo; Brown Nancy V; Addo Marylyn M; Shau Hungyi; Lieberman Judy; Luster Andrew D; Walker Bruce D. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, USA.. acceleration@rcn.com) . Journal of biological chemistry, (2003 Jan 17) 278 (3) 1569-74. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB CD8(+) T-cells are a major source for the production of non-cytolytic factors that inhibit **HIV**-1 replication. In order to characterize further these factors, we analyzed gene expression profiles of activated CD8(+) T-cells using a human cDNA expression array containing 588 human cDNAs. mRNA for the chemokine I-309 (CCL1), the cytokines granulocyte-macrophage colony-stimulating factor and interleukin-13, and natural killer cell enhancing factors (**NKEF**) -A and -B were up-regulated in bulk CD8(+) T-cells from **HIV**-1 seropositive individuals compared with seronegative individuals. Recombinant **NKEF**-A and **NKEF**-B inhibited **HIV**-1 replication when exogenously added to acutely infected T-cells at an ID(50) (dose inhibiting **HIV**-1 replication by 50%) of approximately 130 nm (3 microg/ml). Additionally, inhibition against dual-tropic simian immunodeficiency virus and dual-tropic simian-**human immunodeficiency virus** was found. T-cells transfected with **NKEF**-A or **NKEF**-B cDNA were able to inhibit 80-98% **HIV**-1 replication in vitro. Elevated plasma levels of both **NKEF**-A and **NKEF**-B proteins were detected in 23% of **HIV**-infected non-treated individuals but not in persons treated with highly active antiviral therapy or uninfected persons. These results indicate that the **peroxiredoxin** family members **NKEF**-A and **NKEF**-B are up-regulated in activated CD8(+) T-cells in **HIV** infection, and suggest that these antioxidant proteins contribute to the antiviral activity of CD8(+) T-cells.

L20 ANSWER 2 OF 3 MEDLINE on STN
2001337108. PubMed ID: 11148007. Drug targets and mechanisms of resistance in the anaerobic protozoa. Upcroft P; Upcroft J A. (Queensland Institute of Medical Research and The Tropical Health Program, Australian Centre for International and Tropical Health and Nutrition, The University of Queensland, The Bancroft Centre, Brisbane, Queensland 4029, Australia.. peterU@qimr.edu.au) . Clinical microbiology reviews, (2001 Jan) 14 (1) 150-64. Ref: 244. Journal code: 8807282. ISSN: 0893-8512. Pub. country: United States. Language: English.

AB The anaerobic protozoa Giardia duodenalis, Trichomonas vaginalis, and Entamoeba histolytica infect up to a billion people each year. G. duodenalis and E. histolytica are primarily pathogens of the intestinal tract, although E. histolytica can form abscesses and invade other organs, where it can be fatal if left untreated. T. vaginalis infection is a sexually transmitted infection causing vaginitis and acute inflammatory disease of the genital mucosa. T. vaginalis has also been reported in the urinary tract, fallopian tubes, and pelvis and can cause pneumonia, bronchitis, and oral lesions. Respiratory infections can be acquired perinatally. T. vaginalis infections have been associated with preterm

delivery, low birth weight, and increased mortality as well as predisposing to **human immunodeficiency virus** infection, AIDS, and cervical cancer. All three organisms lack mitochondria and are susceptible to the nitroimidazole metronidazole because of similar low-redox-potential anaerobic metabolic pathways. Resistance to metronidazole and other drugs has been observed clinically and in the laboratory. Laboratory studies have identified the enzyme that activates metronidazole, pyruvate:ferredoxin oxidoreductase, to its nitroso form and distinct mechanisms of decreasing drug susceptibility that are induced in each organism. Although the nitroimidazoles have been the drug family of choice for treating the anaerobic protozoa, G. duodenalis is less susceptible to other antiparasitic drugs, such as furazolidone, albendazole, and quinacrine. Resistance has been demonstrated for each agent, and the mechanism of resistance has been investigated. Metronidazole resistance in T. vaginalis is well documented, and the principal mechanisms have been defined. Bypass metabolism, such as alternative oxidoreductases, have been discovered in both organisms. Aerobic versus anaerobic resistance in T. vaginalis is discussed. Mechanisms of metronidazole resistance in E. histolytica have recently been investigated using laboratory-induced resistant isolates. Instead of downregulation of the pyruvate:ferredoxin oxidoreductase and ferredoxin pathway as seen in G. duodenalis and T. vaginalis, E. histolytica induces oxidative stress mechanisms, including superoxide dismutase and **peroxiredoxin**. The review examines the value of investigating both clinical and laboratory-induced syngeneic drug-resistant isolates and dissection of the complementary data obtained. Comparison of resistance mechanisms in anaerobic bacteria and the parasitic protozoa is discussed as well as the value of studies of the epidemiology of resistance.

L20 ANSWER 3 OF 3 MEDLINE on STN

2001194577. PubMed ID: 11233141. From cytoprotection to tumor suppression: the multifactorial role of **peroxiredoxins**. Butterfield L H; Merino A; Golub S H; Shau H. (Division of Surgical Oncology, UCLA School of Medicine, Los Angeles, CA 90095-1782, USA.) Antioxidants & redox signalling, (1999 Winter) 1 (4) 385-402. Ref: 142. Journal code: 100888899. ISSN: 1523-0864. Pub. country: United States. Language: English.

AB In the past decade, a new family of highly conserved antioxidant enzymes, **Peroxiredoxins** (**Prxs**), have been discovered and defined. There are two major **Prx** subfamilies: one subfamily uses two conserved cysteines (2-Cys) and the other uses 1-Cys to scavenge reactive oxygen species (ROS). This review focuses on the four mammalian 2-Cys members (**Prx** I-IV) that utilize thioredoxin as the electron donor for antioxidation. The array of biological activities of these proteins suggests that they may be evolutionarily important for cell function. For example, **Prxs** are capable of protecting cells from ROS insult and regulating the signal transduction pathways that utilize c-Abl, caspases, nuclear factor-kappaB (NF-kappaB) and activator protein-1 (AP-1) to influence cell growth and apoptosis. **Prxs** are also essential for red blood cell (RBC) differentiation and are capable of inhibiting **human immunodeficiency virus** (**HIV**) infection and organ transplant rejection. Distribution patterns indicate that **Prxs** are highly expressed in the tissues and cells at risk for diseases related to ROS toxicity, such as Alzheimer's and Parkinson's diseases and atherosclerosis. This interesting correlation suggests that **Prxs** are protective against ROS toxicity, yet overwhelmed by oxidative stress in some cells. **Prxs** tend to form large aggregates at high concentrations, a feature that may interfere with their normal protective function or may even render them cytotoxic. Imbalance in the expression of subtypes can also potentially increase their susceptibility to oxidative stress. Understanding the function and biological role of **Prxs** may lead to important discoveries about the cellular dysfunction of ROS-related diseases ranging from atherosclerosis to cancer to neurodegenerative diseases.

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     (FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

     FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
                E LYNN RALF GEIBEN/IN
L1              1 S E3
                E WALKER BRUCE D/IN
L2              7 S E3
L3              6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
                E LYNN R G/IN
L4              2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
                E LYNN R G/AU
                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5              5 S E3 OR E4
                E WALKER B D/AU
L6            155 S E3
L7            154 S L6 NOT L5
L8              0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9            127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10           101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11             1 S E2
L12           526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13           120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14           120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15            11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16            10 S L15 NOT (L1 OR L11)
L17           109 S L13 NOT L15
L18            54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19           535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20             3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l19 and (SIV or simian immunodeficiency virus or lentivir? or retrovir?)
          3765 SIV
         18950 SIMIAN
        112869 IMMUNODEFICIENCY
        373812 VIRUS
          2322 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
          2881 LENTIVIR?
         32204 RETROVIR?
L21             3 L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
                  RETROVIR?)

=> s l21 not l20
L22             2 L21 NOT L20

=> d l22,cbib,ab,1-2

L22  ANSWER 1 OF 2     MEDLINE on STN
2003287224.    PubMed ID: 12815311.   Retrovirally mediated overexpression of
     peroxiredoxin VI increases the survival of WI-38 human diploid
     fibroblasts exposed to cytotoxic doses of tert-butylhydroperoxide and UVB.
     Dierick Jean-Francois; Wenders Frederic; Chainiaux Florence; Remacle Jose;
```

University of Namur (FUNDP), Rue de Bruxelles 61, B-5000 Namur, Belgium.)
Biogerontology, (2003) 4 (3) 125-31. Journal code: 100930043. ISSN: 1389-5729. Pub. country: Netherlands. Language: English.

AB In this work, stable overexpression of **peroxiredoxin** VI was generated in WI-38 human diploid fibroblasts using a **retrovirus**-mediated transfection system. Estimation of cell survival showed that **peroxiredoxin** VI provides a significant protection against tert-butylhydroperoxide- or UVB-caused cytotoxicity. No protection was found against ethanol- or H(2)O(2)-caused cytotoxicity. These effects are correlated with the known functions of **Prx** VI.

L22 ANSWER 2 OF 2 MEDLINE on STN

2002682902. PubMed ID: 12444601. Induction of radioprotective **peroxiredoxin**-I by ionizing irradiation. Chen Wen-Cheng; McBride William H; Iwamoto Keisuke S; Barber Chad L; Wang Chun-Chieh; Oh Young-Taek; Liao Yu-Pei; Hong Ji-Hong; de Vellis Jean; Shau Hungyi. (Department of Radiation Oncology, Chang Gung Memorial Hospital, Chia-Yi, Taiwan.) Journal of neuroscience research, (2002 Dec 15) 70 (6) 794-8. Journal code: 7600111. ISSN: 0360-4012. Pub. country: United States. Language: English.

AB Results of this study indicate a radioprotective effect of **peroxiredoxin**-I. **Peroxiredoxin**-I is an antioxidant that scavenges hydroperoxides, whereas reactive oxygen species are the main mediators of ionizing radiation toxicity. We hypothesized that **peroxiredoxin**-I might be induced by cellular exposure to radiation and act to protect them against its cytotoxic effects. Western blot and Northern blot analyses were used to assess **peroxiredoxin**-I protein and mRNA expression. Rat C6 glioma cells were engineered to overexpress sense or antisense human **peroxiredoxin**-I using **retroviral** vectors. Clonogenic cell survival was used to assess radiosensitivities of the engineered cells. Ionizing radiation induced **peroxiredoxin**-I protein and mRNA expression in human HT29 colon cancer and rat C6 glioma cells in a dose- and time-dependent manner over a 24 hr period. To determine the effect of **peroxiredoxin**-I on radiation responses, C6 glioma cells were engineered to overexpress sense or antisense human **peroxiredoxin**-I. In clonogenic assays, cells overexpressing **peroxiredoxin**-I were more radioresistant. Cells transduced with antisense **peroxiredoxin**-I were marginally more sensitive to radiation toxicity. Irradiation can induce **peroxiredoxin**-I expression, and the increased **peroxiredoxin**-I may protect cells from further radiation damage. These results suggest that protection by **peroxiredoxin**-I may play an important role in the survival of glioma and colon cancer cells in patients undergoing radiation therapy.
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L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

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E LYNN R G/IN
L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
E LYNN R G/AU
E LYNN RALF G/AU

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                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4
                E WALKER B D/AU
L6          155 S E3
L7          154 S L6 NOT L5
L8            0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9          127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10         101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
L12         526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13         120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14         120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15          11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16          10 S L15 NOT (L1 OR L11)
L17         109 S L13 NOT L15
L18          54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19         535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20           3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21           3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22           2 S L21 NOT L20

=> e butterfield l h/au
E1            1     BUTTERFIELD L B/AU
E2            5     BUTTERFIELD L C/AU
E3           23 --> BUTTERFIELD L H/AU
E4           38     BUTTERFIELD L J/AU
E5            1     BUTTERFIELD L M/AU
E6            1     BUTTERFIELD L O/AU
E7            1     BUTTERFIELD LAURA/AU
E8           10     BUTTERFIELD LISA H/AU
E9           18     BUTTERFIELD M/AU
E10           1     BUTTERFIELD M A/AU
E11           4     BUTTERFIELD M C/AU
E12          11     BUTTERFIELD M I/AU

=> s e3
L23          23 "BUTTERFIELD L H"/AU

=> d l23,ti,1-23

L23  ANSWER 1 OF 23     MEDLINE on STN
TI   CD40 cross-linking bypasses the absolute requirement for CD4 T cells
     during immunization with melanoma antigen gene-modified dendritic cells.

L23  ANSWER 2 OF 23     MEDLINE on STN
TI   alpha-Fetoprotein-specific tumor immunity induced by plasmid
     prime-adenovirus boost genetic vaccination.

L23  ANSWER 3 OF 23     MEDLINE on STN
TI   T cell responses to HLA-A*0201-restricted peptides derived from human
     alpha fetoprotein.

L23  ANSWER 4 OF 23     MEDLINE on STN
TI   Fine specificity analysis of an HLA-A2.1-restricted immunodominant T cell
     epitope derived from human alpha-fetoprotein.

L23  ANSWER 5 OF 23     MEDLINE on STN
TI   Immunosensitization of melanoma tumor cells to non-MHC Fas-mediated
     killing by MART-1-specific CTL cultures.
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L23 ANSWER 6 OF 23 MEDLINE on STN
TI From cytoprotection to tumor suppression: the multifactorial role of peroxiredoxins.

L23 ANSWER 7 OF 23 MEDLINE on STN
TI Adenovirus-interleukin-12-mediated tumor regression in a murine hepatocellular carcinoma model is not dependent on CD1-restricted natural killer T cells.

L23 ANSWER 8 OF 23 MEDLINE on STN
TI Genetic immunotherapy for cancer.

L23 ANSWER 9 OF 23 MEDLINE on STN
TI Immune deviation and Fas-mediated deletion limit antitumor activity after multiple dendritic cell vaccinations in mice.

L23 ANSWER 10 OF 23 MEDLINE on STN
TI Generation of T-cell immunity to a murine melanoma using MART-1-engineered dendritic cells.

L23 ANSWER 11 OF 23 MEDLINE on STN
TI Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication.

L23 ANSWER 12 OF 23 MEDLINE on STN
TI Characterization of antitumor immunization to a defined melanoma antigen using genetically engineered murine dendritic cells.

L23 ANSWER 13 OF 23 MEDLINE on STN
TI p53 selective and nonselective replication of an E1B-deleted adenovirus in hepatocellular carcinoma.

L23 ANSWER 14 OF 23 MEDLINE on STN
TI Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein.

L23 ANSWER 15 OF 23 MEDLINE on STN
TI Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma.

L23 ANSWER 16 OF 23 MEDLINE on STN
TI Antitumor protection using murine dendritic cells pulsed with acid-eluted peptides from in vivo grown tumors of different immunogenicities.

L23 ANSWER 17 OF 23 MEDLINE on STN
TI Generation of CD8+ and CD4+ T-cell response to dendritic cells genetically engineered to express the MART-1/Melan-A gene.

L23 ANSWER 18 OF 23 MEDLINE on STN
TI Generation of melanoma-specific cytotoxic T lymphocytes by dendritic cells transduced with a MART-1 adenovirus.

L23 ANSWER 19 OF 23 MEDLINE on STN
TI In vivo therapy of hepatocellular carcinoma with a tumor-specific adenoviral vector expressing interleukin-2.

L23 ANSWER 20 OF 23 MEDLINE on STN
TI Genetic immunization for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells.

L23 ANSWER 21 OF 23 MEDLINE on STN
TI Cloning and analysis of MART-1/Melan-A human melanoma antigen promoter regions.

L23 ANSWER 22 OF 23 MEDLINE on STN

TI A comparison of gene transfer methods in human dendritic cells.

L23 ANSWER 23 OF 23 MEDLINE on STN
TI Cloning and sequence analysis of candidate human natural killer-enhancing factor genes.

=> e shau h/au
E1 1 SHAU ER I/AU
E2 1 SHAU FONG K/AU
E3 53 --> SHAU H/AU
E4 9 SHAU H Y/AU
E5 3 SHAU HUNGYI/AU
E6 1 SHAU M/AU
E7 1 SHAU MIN DA/AU
E8 1 SHAU S E/AU
E9 1 SHAU V/AU
E10 27 SHAU W Y/AU
E11 16 SHAU WEN YI/AU
E12 10 SHAU Y W/AU

=> s e3-e5
53 "SHAU H"/AU
9 "SHAU H Y"/AU
3 "SHAU HUNGYI"/AU
L24 65 ("SHAU H"/AU OR "SHAU H Y"/AU OR "SHAU HUNGYI"/AU)

=> d l24,ti,1-65

L24 ANSWER 1 OF 65 MEDLINE on STN
TI HIV-1 antiviral activity of recombinant natural killer cell enhancing factors, NKEF-A and NKEF-B, members of the peroxiredoxin family.

L24 ANSWER 2 OF 65 MEDLINE on STN
TI Expression of the p75 TNF receptor is linked to TNF-induced NFkappaB translocation and oxyradical neutralization in glial cells.

L24 ANSWER 3 OF 65 MEDLINE on STN
TI Induction of radioprotective peroxiredoxin-I by ionizing irradiation.

L24 ANSWER 4 OF 65 MEDLINE on STN
TI Induction of peroxiredoxins in transplanted livers and demonstration of their in vitro cytoprotection activity.

L24 ANSWER 5 OF 65 MEDLINE on STN
TI Contrasting antioxidant and cytotoxic effects of peroxiredoxin I and II in PC12 and NIH3T3 cells.

L24 ANSWER 6 OF 65 MEDLINE on STN
TI From cytoprotection to tumor suppression: the multifactorial role of peroxiredoxins.

L24 ANSWER 7 OF 65 MEDLINE on STN
TI Differential expression of peroxiredoxin subtypes in human brain cell types.

L24 ANSWER 8 OF 65 MEDLINE on STN
TI Oxidative stress produced by marijuana smoke. An adverse effect enhanced by cannabinoids.

L24 ANSWER 9 OF 65 MEDLINE on STN
TI Expression of the antioxidant gene NKEF in the central nervous system.

L24 ANSWER 10 OF 65 MEDLINE on STN
TI Thioredoxin peroxidase (natural killer enhancing factor) regulation of activator protein-1 function in endothelial cells.

L24 ANSWER 11 OF 65 MEDLINE on STN
TI Characterization of antioxidant properties of natural killer-enhancing factor-B and induction of its expression by hydrogen peroxide.

L24 ANSWER 12 OF 65 MEDLINE on STN
TI Pravastatin increases survival and inhibits natural killer cell enhancement factor in liver transplanted rats.

L24 ANSWER 13 OF 65 MEDLINE on STN
TI Cellular antioxidant properties of human natural killer enhancing factor B.

L24 ANSWER 14 OF 65 MEDLINE on STN
TI Endogenous natural killer enhancing factor-B increases cellular resistance to oxidative stresses.

L24 ANSWER 15 OF 65 MEDLINE on STN
TI Human thymocyte responsiveness to stem cell factor: synergy with interleukin-2 for the generation of NK/LAK cytotoxicity.

L24 ANSWER 16 OF 65 MEDLINE on STN
TI Recombinant natural killer enhancing factor augments natural killer cytotoxicity.

L24 ANSWER 17 OF 65 MEDLINE on STN
TI Characterization of an oxidation-resistant tumor cell line and its sensitivity to immune response and chemotherapy.

L24 ANSWER 18 OF 65 MEDLINE on STN
TI Antioxidant function of recombinant human natural killer enhancing factor.

L24 ANSWER 19 OF 65 MEDLINE on STN
TI Cloning and sequence analysis of candidate human natural killer-enhancing factor genes.

L24 ANSWER 20 OF 65 MEDLINE on STN
TI A comparative study of intravenous versus intralymphatic interleukin-2, with assessment of effects of interleukin-2 on both peripheral blood and thoracic-duct lymph.

L24 ANSWER 21 OF 65 MEDLINE on STN
TI Role of enhanced cellular adhesion in IL-6-augmented lymphokine-activated killer-cell function.

L24 ANSWER 22 OF 65 MEDLINE on STN
TI Identification of natural killer enhancing factor as a major antioxidant in human red blood cells.

L24 ANSWER 23 OF 65 MEDLINE on STN
TI Effect of ASTA-Z 7575 (INN Maphosphamide) on human lymphokine-activated killer cell induction.

L24 ANSWER 24 OF 65 MEDLINE on STN
TI Transduction of human melanoma cell lines with the human interleukin-7 gene using retroviral-mediated gene transfer: comparison of immunologic properties with interleukin-2.

L24 ANSWER 25 OF 65 MEDLINE on STN
TI Regulation of natural killer function by nonlymphoid cells.

L24 ANSWER 26 OF 65 MEDLINE on STN
TI Pulmonary surfactant inhibits interleukin-2-induced proliferation and the generation of lymphokine-activated killer cells.

L24 ANSWER 27 OF 65 MEDLINE on STN

TI [illegible] variety of lymphoid tissues.

L24 ANSWER 28 OF 65 MEDLINE on STN
TI Interferon-alpha primed tumor-infiltrating lymphocytes combined with interleukin-2 and interferon-alpha as therapy for metastatic renal cell carcinoma.

L24 ANSWER 29 OF 65 MEDLINE on STN
TI Interleukin-6 is a mediator of TNF-alpha regulation of LAK cell function.

L24 ANSWER 30 OF 65 MEDLINE on STN
TI Identification of a natural killer enhancing factor (NKEF) from human erythroid cells.

L24 ANSWER 31 OF 65 MEDLINE on STN
TI IL-6 enhances the cytotoxic activity of thymocyte-derived CD56+ cells.

L24 ANSWER 32 OF 65 MEDLINE on STN
TI Tumor necrosis factor stimulation of neutrophils for antitumor activity.

L24 ANSWER 33 OF 65 MEDLINE on STN
TI Protein A potentiates lymphokine-activated killer cell induction in normal and melanoma patient lymphocytes.

L24 ANSWER 34 OF 65 MEDLINE on STN
TI Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin.

L24 ANSWER 35 OF 65 MEDLINE on STN
TI Effects of tumor-necrosis-factor-activated neutrophils on tumor cell survival.

L24 ANSWER 36 OF 65 MEDLINE on STN
TI Red cell regulation of tumor necrosis factor-induced human neutrophil cytostatic activity.

L24 ANSWER 37 OF 65 MEDLINE on STN
TI The effects of staphylococcal protein A on human lymphokine-activated killer cell induction.

L24 ANSWER 38 OF 65 MEDLINE on STN
TI Characteristics of interleukin-6-enhanced lymphokine-activated killer cell function.

L24 ANSWER 39 OF 65 MEDLINE on STN
TI IL-1 and IL-4 as reciprocal regulators of IL-2 induced lymphocyte cytotoxicity.

L24 ANSWER 40 OF 65 MEDLINE on STN
TI Increased lysis of melanoma by in vivo-elicited human lymphokine-activated killer cells after addition of antiganglioside antibodies in vitro.

L24 ANSWER 41 OF 65 MEDLINE on STN
TI A pilot study of intralymphatic interleukin-2. I. Cytotoxic and surface marker changes of peripheral blood lymphocytes.

L24 ANSWER 42 OF 65 MEDLINE on STN
TI Low-dose cyclophosphamide and low-dose interleukin-2 for malignant melanoma.

L24 ANSWER 43 OF 65 MEDLINE on STN
TI Inhibition of lymphokine-activated killer- and natural killer-mediated cytotoxicities by neutrophils.

L24 ANSWER 44 OF 65 MEDLINE on STN

TI Suppression of lymphokine activated killer induction by neutrophils.

L24 ANSWER 45 OF 65 MEDLINE on STN
TI Generation of lymphokine-activated killer cell activity from non-NK precursor cells.

L24 ANSWER 46 OF 65 MEDLINE on STN
TI Studies on cytotoxicity generated in human mixed lymphocyte culture. IV. Interleukin 2 alone or from mixed lymphocyte culture yields natural killer-like cytotoxic cells distinct from allospecific cytotoxic T lymphocytes.

L24 ANSWER 47 OF 65 MEDLINE on STN
TI Modulation of natural killer-mediated lysis by red blood cells.

L24 ANSWER 48 OF 65 MEDLINE on STN
TI Active specific immunotherapy for melanoma: phase I trial of allogeneic lysates and a novel adjuvant.

L24 ANSWER 49 OF 65 MEDLINE on STN
TI Studies on the relationship of human natural killer and lymphokine-activated killer cells with lysosomal staining and analysis of surface marker phenotypes.

L24 ANSWER 50 OF 65 MEDLINE on STN
TI Characteristics and mechanism of neutrophil-mediated cytostasis induced by tumor necrosis factor.

L24 ANSWER 51 OF 65 MEDLINE on STN
TI Role of proliferation in LAK cell development.

L24 ANSWER 52 OF 65 MEDLINE on STN
TI Effectiveness and tolerability of low-dose cyclophosphamide and low-dose intravenous interleukin-2 in disseminated melanoma [corrected].

L24 ANSWER 53 OF 65 MEDLINE on STN
TI Cytostatic and tumoricidal activities of tumor necrosis factor-treated neutrophils.

L24 ANSWER 54 OF 65 MEDLINE on STN
TI Lysosome rich cells contain the lytic activity of lymphokine-activated killer cell populations.

L24 ANSWER 55 OF 65 MEDLINE on STN
TI The role of transferrin in natural killer cell and IL-2-induced cytotoxic cell function.

L24 ANSWER 56 OF 65 MEDLINE on STN
TI Functional studies on the precursors of human lymphokine-activated killer cells.

L24 ANSWER 57 OF 65 MEDLINE on STN
TI Signals for activation of natural killer and natural killer-like activity.

L24 ANSWER 58 OF 65 MEDLINE on STN
TI Immunological aspects of retinoids in humans. III. Effects of retinoic acid on the natural killing of tumor cells.

L24 ANSWER 59 OF 65 MEDLINE on STN
TI Regulation of human natural killing by lysosomotropic and thiol-reactive agents.

L24 ANSWER 60 OF 65 MEDLINE on STN
TI Identification and purification of NK cells with lysosomotropic vital stains: correlation of lysosome content with NK activity.

L24 ANSWER 61 OF 65 MEDLINE on STN
TI Depletion of NK cells with the lysosomotropic agent L-leucine methyl ester and the in vitro generation of NK activity from NK precursor cells.

L24 ANSWER 62 OF 65 MEDLINE on STN
TI The role of the lysosome in natural killing: inhibition by lysosomotropic vital dyes.

L24 ANSWER 63 OF 65 MEDLINE on STN
TI The humoral response to gynecologic malignancies and its role in the regulation of tumor growth: a review.

L24 ANSWER 64 OF 65 MEDLINE on STN
TI Human natural killing against ovarian carcinoma.

L24 ANSWER 65 OF 65 MEDLINE on STN
TI Regulation of human natural killing by levamisole.

=> d l24,cbib,ab,1,9-11,13,14

L24 ANSWER 1 OF 65 MEDLINE on STN
2003032310. PubMed ID: 12421812. HIV-1 antiviral activity of recombinant natural killer cell enhancing factors, NKEF-A and NKEF-B, members of the peroxiredoxin family. Geiben-Lynn Ralf; Kursar Mischo; Brown Nancy V; Addo Marylyn M; **Shau Hungyi**; Lieberman Judy; Luster Andrew D; Walker Bruce D. (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, USA.. acceleration@rcn.com) . Journal of biological chemistry, (2003 Jan 17) 278 (3) 1569-74. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB CD8(+) T-cells are a major source for the production of non-cytolytic factors that inhibit HIV-1 replication. In order to characterize further these factors, we analyzed gene expression profiles of activated CD8(+) T-cells using a human cDNA expression array containing 588 human cDNAs. mRNA for the chemokine I-309 (CCL1), the cytokines granulocyte-macrophage colony-stimulating factor and interleukin-13, and natural killer cell enhancing factors (NKEF) -A and -B were up-regulated in bulk CD8(+) T-cells from HIV-1 seropositive individuals compared with seronegative individuals. Recombinant NKEF-A and NKEF-B inhibited HIV-1 replication when exogenously added to acutely infected T-cells at an ID(50) (dose inhibiting HIV-1 replication by 50%) of approximately 130 nm (3 microg/ml). Additionally, inhibition against dual-tropic simian immunodeficiency virus and dual-tropic simian-human immunodeficiency virus was found. T-cells transfected with NKEF-A or NKEF-B cDNA were able to inhibit 80-98% HIV-1 replication in vitro. Elevated plasma levels of both NKEF-A and NKEF-B proteins were detected in 23% of HIV-infected non-treated individuals but not in persons treated with highly active antiviral therapy or uninfected persons. These results indicate that the peroxiredoxin family members NKEF-A and NKEF-B are up-regulated in activated CD8(+) T-cells in HIV infection, and suggest that these antioxidant proteins contribute to the antiviral activity of CD8(+) T-cells.

L24 ANSWER 9 OF 65 MEDLINE on STN
1998451667. PubMed ID: 9778645. Expression of the antioxidant gene NKEF in the central nervous system. Sarafian T A; Huang C; Kim A; de Vellis J; **Shau H**. (Department of Pathology and Experimental Medicine, UCLA School of Medicine 90095, USA.) Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid, (1998 May) 34 (1) 39-51. Journal code: 8910358. ISSN: 1044-7393. Pub. country: United States. Language: English.
AB Free radicals and the oxidative stress they impose can cause serious injury in the nervous system and contribute to pathology associated with a wide variety of degenerative and traumatic disorders. In this study, we

examined the expression of an antioxidant defense gene, NKEF, in human tissue and isolated populations of rat brain cells using Western and Northern blot analysis. NKEF protein was expressed in human brain, liver, kidney, muscle, and lung. The human endothelial cell line ECV expressed a 25-kDa band in addition to the 22-kDa band normally observed. In the central nervous system, a 22-kDa NKEF band was present in cortical gray and white matter, hippocampus, cerebellum, and spinal cord in roughly similar amounts. Expression of NKEF-A and NKEF-B subtypes was evaluated by Northern analysis of cultured cell types from embryonic rat brain. Astrocyte and microglia expressed both 22- and 25-kDa bands, whereas cortical neurons and oligodendrocytes contained only the 22-kDa protein band. Northern blot analysis of these cell types revealed low levels of NKEF-A message in neurons and oligodendrocytes, and relatively low levels of NKEF-B in microglia. Differential expression of these antioxidant defense genes may contribute to the selective vulnerability of brain cell types to specific kinds of oxidative stress.

L24 ANSWER 10 OF 65 MEDLINE on STN

1998401150. PubMed ID: 9731197. Thioredoxin peroxidase (natural killer enhancing factor) regulation of activator protein-1 function in endothelial cells. **Shau H**; Huang A C; Faris M; Nazarian R; de Vellis J; Chen W. (Division of Surgical Oncology, UCLA School of Medicine 90095, USA.. hshau@ucla.edu) . Biochemical and biophysical research communications, (1998 Aug 28) 249 (3) 683-6. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Thioredoxin peroxidase-1 (TxP-1), originally cloned as natural killer enhancing factor-B, belongs to a highly conserved antioxidant family. Tumor necrosis factor-alpha (TNF) activates the expression of activator protein-1 (AP-1) responsive genes. We show here that over-expression of TxP-1 blocks TNF-induced AP-1 activation in endothelial ECV304 cells, which was demonstrated by three independent experimental protocols: electromobility shift assay with AP-1 oligonucleotide probe; reporter gene expression with AP-1 binding site, and interleukin-8 production, which is dependent on AP-1. These results are consistent with the role of TxP-1 as an antioxidant and the previous reports that TNF-induced reactive oxygen species were responsible for AP-1 activation.

L24 ANSWER 11 OF 65 MEDLINE on STN

1998023023. PubMed ID: 9356316. Characterization of antioxidant properties of natural killer-enhancing factor-B and induction of its expression by hydrogen peroxide. Kim A T; Sarafian T A; **Shau H**. (Division of Surgical Oncology, UCLA School of Medicine, Los Angeles, California 90095, USA.) Toxicology and applied pharmacology, (1997 Nov) 147 (1) 135-42. Journal code: 0416575. ISSN: 0041-008X. Pub. country: United States. Language: English.

AB Natural killer-enhancing factor B (NKEF-B) belongs to a highly conserved family of recently discovered antioxidants. The role of NKEF-B as an antioxidant was demonstrated by its protection of transfected cells to oxidative damage by hydrogen peroxide. To further characterize the antioxidant properties of NKEF-B, we compared the sensitivity of a human endothelial cell line ECV304 and its transfectant, B/1 that hyperexpresses NKEF-B, to various oxidants. In addition, we investigated the changes in the expression of NKEF-B mRNA upon oxidative stress. We found that B/1 was significantly more resistant than the control cells to the oxidative stresses caused by t-butyl hydroperoxide (t-BHP) and methyl mercury (MeHg). In contrast, there was no difference in the sensitivity of B/1 and the control cells to sulfhydryl reactive agents, diethyl maleate and diamide. B/1 was also as sensitive as the control cells to buthionine sulfoximine. The expression of NKEF-B mRNA was induced when the parental cell line ECV304 was treated with 2 mm HP. The induction reached a maximum level around 2 hr and decreased to the basal level around 4 hr. NKEF-A mRNA was not induced by HP. These results demonstrate antioxidant activities of NKEF-B toward prooxidants such as alkyl hydroperoxide and MeHg. Together with its antioxidant activity, the induction of NKEF-B by HP indicates that NKEF-B is an important oxidative stress protein providing protection against a variety of xenobiotic toxic agents.

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L24 ANSWER 13 OF 65 MEDLINE on STN

97305591. PubMed ID: 9161849. Cellular antioxidant properties of human natural killer enhancing factor B. Sarafian T A; Rajper N; Grigorian B; Kim A; **Shau H.** (Department of Pathology, UCLA 90095, USA.) Free radical research, (1997 Mar) 26 (3) 281-9. Journal code: 9423872. ISSN: 1071-5762. Pub. country: Switzerland. Language: English.

AB The protein, NKEF (natural killer enhancing factor), has been identified as a member of an antioxidant family of proteins capable of protecting against protein oxidation in cell-free assay systems. The mechanism of action for this family of proteins appears to involve scavenging or suppressing formation of protein thiyl radicals. In the present study we investigated the antioxidant protective properties of the NKEF-B protein overexpressed in an endothelial cell line (ECV304). Nkef-B-transfected cells displayed significantly lower levels of reactive oxygen species (ROS) compared with control or vector-transfected cells. Tert-Butylhydroperoxide-induced ROS was 15% lower in nkef-B-transfected cells and cytotoxicity was slightly, though not significantly, lower. NKEF-B had no effect on ROS induced by menadione or xanthine plus xanthine oxidase. NKEF-B overexpression resulted in slightly (approximately 10%) lower levels of cellular glutathione (GSH) and had no effect on rate or extent of GSH depletion following either diethylmaleate (DEM) or buthionine sulfoximine (BSO) treatment. Lipid peroxidation, assessed as thiobarbituric acid-reactive substances, was 40% lower in nkef-B-transfected cells compared with vector-only-transfected cells. DEM-induced lipid peroxidation was suppressed by NKEF-B at DEM concentrations of 20 microM to 1 mM. At 10 mM DEM, lipid peroxidation was unaffected by NKEF-B. NKEF-B expression also protected cells against menadione-induced inhibition of [3H]-thymidine uptake. The NKEF-B protein appears most effective in suppressing basal low-level oxidative injury such as that produced during normal metabolism. These results indicate that overexpression of the NKEF-B protein promotes resistance to oxidative stress in this endothelial cell line.

L24 ANSWER 14 OF 65 MEDLINE on STN

97135505. PubMed ID: 8981042. Endogenous natural killer enhancing factor-B increases cellular resistance to oxidative stresses. **Shau H;** Kim A T; Hedrick C C; Lusis A J; Tompkins C; Finney R; Leung D W; Paglia D E. (Division of Surgical Oncology, UCLA Medical Center 90095, USA.) Free radical biology & medicine, (1997) 22 (3) 497-507. Journal code: 8709159. ISSN: 0891-5849. Pub. country: United States. Language: English.

AB Natural killer-enhancing factor (NKEF) was identified and cloned on the basis of its ability to increase NK cytotoxicity. Two genes, NKEF-A and -B, encode NKEF proteins and sequence analysis presented suggests that each belongs to a highly conserved family of antioxidants. To examine the antioxidant potential of NKEF, we transfected the coding region of NKEF-B cDNA into the human endothelial cell line ECV304. The stable transfectant, B/1, was found to overexpress NKEF-B gene transcript and protein. We subjected B/1 to oxidative stress by either culturing them with glucose oxidase (GO), which continuously generates hydrogen peroxide, or by direct addition of hydrogen peroxide. We found that B/1 cells were more resistant than control cell lines. Resistance to hydrogen peroxide was originally thought to be mediated mainly by catalase and the glutathione cycle. Therefore, we used inhibitors to block the two pathways and found that B/1 cells were more resistant to oxidative stress than control cells when we used inhibitors to preblock either pathway. We also examined the cellular inflammatory responses to oxidized low-density lipoprotein (LDL) and bacterial lipopolysaccharide (LPS) by measuring monocyte adhesion to endothelial cells in vitro and found that B/1 cells were resistant to such responses. Lastly, we found that B/1 cells were more resistant to a novel chemotherapeutic agent CT-2584, which appears to kill tumor cells by stimulating production of reactive oxygen intermediates in mitochondria. These results demonstrate that the NKEF-B is an antioxidant that protects cells from oxidative stress, chemotherapy agents, and inflammation-induced monocyte adhesion. Furthermore, its

expression may modulate cellular responses to proinflammatory molecules.

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(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
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L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
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L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
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E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
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L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

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L11 1 S E2
L12 526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13 120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14 120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
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L16 10 S L15 NOT (L1 OR L11)
L17 109 S L13 NOT L15
L18 54 S L17 AND AY<2002

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L20 3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21 3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22 2 S L21 NOT L20
E BUTTERFIELD L H/AU
L23 23 S E3
E SHAU H/AU
L24 65 S E3-E5

=> s l19 and (antivir?)
39659 ANTIVIR?
L25 1 L19 AND (ANTIVIR?)

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L25 ANSWER 1 OF 1 MEDLINE on STN
2003032310. PubMed ID: 12421812. HIV-1 **antiviral** activity of recombinant natural killer cell enhancing factors, **NKEF**-A and **NKEF**-B, members of the **peroxiredoxin** family. Geiben-Lynn Ralf; Kursar Mischo; Brown Nancy V; Addo Marylyn M; Shau Hungyi; Lieberman Judy; Luster Andrew D; Walker Bruce D. (Partners AIDS Research Center, Massachusetts General Hospital

and Harvard Medical School, Boston, Massachusetts 02115, USA.
acceleration@rcn.com) . Journal of biological chemistry, (2003 Jan 17) 278 (3) 1569-74. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB CD8(+) T-cells are a major source for the production of non-cytolytic factors that inhibit HIV-1 replication. In order to characterize further these factors, we analyzed gene expression profiles of activated CD8(+) T-cells using a human cDNA expression array containing 588 human cDNAs. mRNA for the chemokine I-309 (CCL1), the cytokines granulocyte-macrophage colony-stimulating factor and interleukin-13, and natural killer cell enhancing factors (**NKEF**) -A and -B were up-regulated in bulk CD8(+) T-cells from HIV-1 seropositive individuals compared with seronegative individuals. Recombinant **NKEF**-A and **NKEF**-B inhibited HIV-1 replication when exogenously added to acutely infected T-cells at an ID(50) (dose inhibiting HIV-1 replication by 50%) of approximately 130 nm (3 microg/ml). Additionally, inhibition against dual-tropic simian immunodeficiency virus and dual-tropic simian-human immunodeficiency virus was found. T-cells transfected with **NKEF**-A or **NKEF**-B cDNA were able to inhibit 80-98% HIV-1 replication in vitro. Elevated plasma levels of both **NKEF**-A and **NKEF**-B proteins were detected in 23% of HIV-infected non-treated individuals but not in persons treated with highly active **antiviral** therapy or uninfected persons. These results indicate that the **peroxiredoxin** family members **NKEF**-A and **NKEF**-B are up-regulated in activated CD8(+) T-cells in HIV infection, and suggest that these antioxidant proteins contribute to the **antiviral** activity of CD8(+) T-cells.

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(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
E LYNN RALF GEIBEN/IN
L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
E LYNN R G/IN
L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
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E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
E WALKER B D/AU
L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
E GEIBEN-LYNN R/IN
L11 1 S E2
L12 526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13 120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14 120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15 11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16 10 S L15 NOT (L1 OR L11)

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L18           54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19          535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20            3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21            3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22            2 S L21 NOT L20
                 E BUTTERFIELD L H/AU
L23           23 S E3
                 E SHAU H/AU
L24           65 S E3-E5
L25            1 S L19 AND (ANTIVIR?)

=> file wpids
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      11.00     134.79

FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
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FILE LAST UPDATED:          29 APR 2004      <20040429/UP>
MOST RECENT DERWENT UPDATE:     200428        <200428/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
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    FOR FURTHER DETAILS:
    http://www.thomsonscientific.com/litalert           <<<

>>> THE DISPLAY LAYOUT HAS BEEN CHANGED TO ACCOMODATE THE
    NEW FORMAT GERMAN PATENT APPLICATION AND PUBLICATION
    NUMBERS. SEE ALSO:
    http://www.stn-international.de/archive/stnews/news0104.pdf <<<

>>> SINCE THE FILE HAD NOT BEEN UPDATED BETWEEN APRIL 12-16
    THERE WAS NO WEEKLY SDI RUN  <<<

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            21 PEROXIREDOXIN?
            10 NKEF?
        158774 NATURAL
          1948 KILLER
         35559 ENHANCING
        149255 FACTOR?
             3 NATURAL KILLER ENHANCING FACTOR?
                 (NATURAL(W)KILLER(W)ENHANCING(W)FACTOR?)
            79 PRX?
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L26           PRX?)

=> s l26 and (antivir? or HIV? or human immunodeficiency virus or lentivir? or retrovir?)
         12243 ANTIVIR?
         18550 HIV?
        143579 HUMAN
          6764 IMMUNODEFICIENCY
         35067 VIRUS
          4477 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
           664 LENTIVIR?
          4315 RETROVIR?
L27          3 L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR
               LENTIVIR? OR RETROVIR?)

=> d his

     (FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

     FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
                E LYNN RALF GEIBEN/IN
L1            1 S E3
                E WALKER BRUCE D/IN
L2            7 S E3
L3            6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
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L4            2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
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                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4
                E WALKER B D/AU
L6          155 S E3
L7          154 S L6 NOT L5
L8            0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9          127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10         101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
L12         526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13         120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14         120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15          11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16          10 S L15 NOT (L1 OR L11)
L17         109 S L13 NOT L15
L18          54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19         535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20           3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21           3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22           2 S L21 NOT L20
                E BUTTERFIELD L H/AU
L23          23 S E3
                E SHAU H/AU
L24          65 S E3-E5
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FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
L26 105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L27 3 S L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR

=> s l27 not l4
L28 2 L27 NOT L4

=> d l28,bib,ab,1-2

L28 ANSWER 1 OF 2 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-198262 [19] WPIDS
CR 2002-351894 [38]; 2003-401356 [38]; 2003-755317 [71]
DNC C2003-050755
TI Composition useful for treating cancer, viral infection and inflammatory conditions such as asthma, comprises a mixture of human cytokines produced by a human cell line which overexpresses a cytokine regulatory factor.
DC B04 D16
IN BROWNING, L; LAU, A S; OSSINA, N; WAN, W H
PA (BROW-I) BROWNING L; (LAUA-I) LAU A S; (OSSI-I) OSSINA N; (WANW-I) WAN W H
CYC 1
PI US 2002150552 A1 20021017 (200319)* 31
ADT US 2002150552 A1 CIP of US 2000-660468 20000912, US 2001-952843 20010911
PRAI US 2001-952843 20010911; US 2000-660468 20000912
AB US2002150552 A UPAB: 20031105
NOVELTY - Composition (I) comprising mixture of human cytokines produced by culturing human cell line capable of producing mixture of cytokines, where the cell line overexpresses cytokine regulatory factor (CF) or anti-apoptotic protein (AP), treating CF- or AP-overexpressing cell line to effect enhanced production of mixture of cytokines, and collecting cytokines produced by the cultured CF- or AP-overexpressing cell line.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for producing (M1) a mixture of human cytokines in a cell culture, which involves culturing a human cell line capable of producing the mixture of cytokines, selecting or modifying the cultured human cell line where a CF is overexpressed by the cell line, treating the cultured, CF-overexpressing cell line to effect cytokine production, and collecting the cytokines produced by the cultured treated cell line.

ACTIVITY - Virucide; Cytostatic; Antiinflammatory; Anti-**HIV**; Antiasthmatic; Antiallergic; Antirheumatic; Antiarthritic.

No biological data given.

MECHANISM OF ACTION - Immune response modulator.

USE - (I) is useful in cancer treatment, where the cytokines produced include two or more cytokines chosen from interleukin-2 (IL-2), IL-12, IL-15, interferon-alpha (IFN- alpha), IFN- beta , IFN- gamma , IFN- omega , tumor necrosis factor-alpha (TNF- alpha), **natural killer enhancing factor** (**NKEF**), natural killer cell stimulatory factor (NKSF), TNF-related-apoptosis-inducing-ligand (TRAIL) and granulocyte macrophage colony-stimulating factor (GM-CSF). (I) is useful in treating viral infection, where the cytokines produced include two or more cytokines chosen from IFN- alpha , IFN- beta , IFN- gamma , IFN- omega , transforming growth factor beta (TGF- beta), IL-8, IL-12 and GM-CSF. (I) is also useful for treating an inflammatory condition, where the cytokines produced include two or more cytokines chosen from IL-4, IL-5, IL-6, IL-10, IFN- beta , IFN- gamma and TGF- beta (claimed). (I) is useful for treating cancer including solid tumors, melanomas, leukemias, and other types of cancers or neoplasms; viral infection including infection by **human immunodeficiency virus** (**HIV**), hepatitis viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV) and other human-pathogenic viruses; and inflammation including asthma, allergies, and rheumatoid arthritis.

ADVANTAGE - (M1) provides increased production of the mixture of human cytokines in cell culture.

Dwg.0/6

L28 ANSWER 2 OF 2 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2001-396982 [42] WPIDS
CR 2000-012791 [01]; 2001-647258 [74]; 2002-690743 [74]
DNC C2001-120711
TI Isolated polynucleotides encoding human natural killer cell enhancing factor C, useful for preventing, diagnosing or treating viral infections, neoplasia and damage from superoxide radicals.
DC B04 D12
IN GENTZ, R; NI, J; ROSEN, C A; YU, G
PA (HUMA-N) HUMAN GENOME SCI INC
CYC 1
PI US 6255079 B1 20010703 (200142)* 49
ADT US 6255079 B1 US 1995-467265 19950606
PRAI US 1995-467265 19950606
AB US 6255079 B UPAB: 20021120
NOVELTY - An isolated nucleic acid molecule (N1) encoding human natural killer cell enhancing factor C is new.

DETAILED DESCRIPTION - (N1) comprises:

(1) a polynucleotide encoding amino acids 1-271 (a), 2-271 (b), or 31-271 (c) of a 271 amino acid sequence (S1) described in the specification;

(2) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA containing in ATCC Deposit No. 97157;

(3) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157;

(4) a polynucleotide encoding at least 30 contiguous amino acids of (S1) or the cDNA clone contained in ATCC Deposit No. 97157;

(5) a polynucleotide encoding at least 50 contiguous amino acids of (S1) or the cDNA clone contained in ATCC Deposit No. 97157;

(6) a polynucleotide of at least 30 contiguous nucleotides of a sequence of 918 nucleotides (S2) described in the specification or the cDNA clone contained in ATCC Deposit No. 97157;

(7) a polynucleotide of at least 50 contiguous nucleotides of a sequence of 918 nucleotides (S2) described in the specification or the cDNA clone contained in ATCC Deposit No. 97157; or

(8) the complement of (1)-(7).

INDEPENDENT CLAIMS are also included for:

(1) a vector (N2) comprising (N1);

(2) a recombinant host (I) comprising (N1) operatively associated with a heterologous regulatory sequence; and

(3) producing a polypeptide comprising:

(a) culturing (I); and

(b) recovering the polypeptide.

ACTIVITY - **Antiviral**; Antiinflammatory; Cytostatic. The cytopathic effect reduction (CPER) assay is employed to measure the protective effect of **NKEF** C on the infection and cytopathic process of vesicular stomatitis virus (VSV) to normal human dermal fibroblasts (NHDF) from foreskin (Clonetics). In this experiment serial dilutions were performed of **NKEF** C at a 1:2 ratio and extended the dilution starting from 3 micro g/ml to 6 ng/ml final concentration. The positive control employed in this experiment was a recombinant human IFN-beta protein, which had a previously determined activity equal to 4x106 units per 100 micro 1. In addition, a negative mock control was used. Semi-purified (about 70%) protein isolated from Escherichia Coli expressing **NKEF** C protein was employed in this study. The NHDF cells were seeded at 2x104 per well and incubated overnight to reach confluence. These cells were incubated for about 12 hours in the presence of each diluted supernatant and then subsequently challenged with VSV at an MOI equal to 1x105 pfu per well. The plates were further incubated for 15 hours and then fixed and stained with crystal violet. The plates were scored for CPE by estimating the percentage of cells surviving on the microtiter plate. The figure in the specification demonstrates a mean effective **NKEF** C concentration equal to about 100 ng/ml.

USE - The invention may be useful in the treatment and/or prevention of viral infections, inflammation, neoplasia and damage from superoxide radicals. The invention is also useful in diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting disease, for example, cancer.
Dwg.0/5

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=> fil ifipat;s (US 2001-952843)/pn,apps and ab/fa
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY     SESSION
FULL ESTIMATED COST                                      33.24      168.03

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FILE COVERS 1950 TO PATENT PUBLICATION DATE: 27 Apr 2004 (20040427/PD)
FILE LAST UPDATED: 28 Apr 2004 (20040428/ED)
HIGHEST GRANTED PATENT NUMBER: US2004064242
HIGHEST APPLICATION PUBLICATION NUMBER: US2004078858
UNITERM INDEXING IS AVAILABLE IN THE IFIUDB FILE
UNITERM INDEXING LAST UPDATED: 30 Apr 2004 (20040430/UP)
INDEXING CURRENT THROUGH PAT PUB DATE: 28 Oct 2003 (20031028/PD)

IFIPAT has been reloaded (12/21/2003).  See HELP RLOAD for details.


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               (US2001952843/PN)
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             0 US2001-952843/PRN
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             3 (US 2001-952843)/APPS
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L29          3 (US 2001-952843)/PN,APPS AND AB/FA

=> fil uspatfull;s (US 2001-952843)/pn,apps
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
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FULL ESTIMATED COST                                       1.61      169.64

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 29 Apr 2004 (20040429/PD)
FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2004
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2004

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>>>  original, i.e., the earliest published granted patents or          <<<
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>>>  publications, starting in 2001, for the inventions covered in      <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original      <<<
>>>  published document but also a list of any subsequent               <<<
>>>  publications.  The publication number, patent kind code, and       <<<
>>>  publication date for all the US publications for an invention      <<<
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                (US2001-952843/AP,PRN,RLN)
L30         3 (US 2001-952843)/PN,APPS

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FULL ESTIMATED COST                                1.35     170.99

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FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
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>>> applications. USPAT2 contains full text of the latest US <<<
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L30 ANSWER 3 OF 3 USPATFULL on STN
```

TI Compositions comprising mixtures of therapeutic proteins and methods of producing the same

AB Human cytokine mixtures produced by cytokine regulatory factor-overexpressing cells and methods of production are disclosed. The mixtures are prepared by culturing human cytokine-producing cells under conditions of cytokine regulatory factor overexpression, treating the cells to induce cytokine production, and isolating the mixtures of cytokines produced by the cells. Exemplary compositions include mixtures of human interferon .gamma. in combination with human interferon .alpha. and/or human interferon .beta., and mixtures of human interferon .alpha. and human interferon .beta.. Also disclosed are therapeutic uses of the interferon compositions.

=> d his

(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
E LYNN RALF GEIBEN/IN
L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
E LYNN R G/IN
L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
E LYNN R G/AU
E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
E WALKER B D/AU
L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
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L11 1 S E2
L12 526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13 120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14 120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15 11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16 10 S L15 NOT (L1 OR L11)
L17 109 S L13 NOT L15
L18 54 S L17 AND AY<2002

FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19 535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20 3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21 3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22 2 S L21 NOT L20
E BUTTERFIELD L H/AU
L23 23 S E3
E SHAU H/AU
L24 65 S E3-E5
L25 1 S L19 AND (ANTIVIR?)

```
L26          105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L27            3 S L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR
L28            2 S L27 NOT L4

     FILE 'IFIPAT' ENTERED AT 12:59:18 ON 02 MAY 2004
L29            3 S (US 2001-952843)/PN,APPS AND AB/FA

     FILE 'USPATFULL' ENTERED AT 12:59:18 ON 02 MAY 2004
L30            3 S (US 2001-952843)/PN,APPS

     FILE 'USPATFULL' ENTERED AT 12:59:38 ON 02 MAY 2004

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     4.97     175.96

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 29 Apr 2004 (20040429/PD)
FILE LAST UPDATED: 29 Apr 2004 (20040429/ED)
HIGHEST GRANTED PATENT NUMBER: US6728968
HIGHEST APPLICATION PUBLICATION NUMBER: US2004083524
CA INDEXING IS CURRENT THROUGH 29 Apr 2004 (20040429/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 29 Apr 2004 (20040429/PD)
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>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
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>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
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>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

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E3            0 --> GENTZ R/IN
E4            1     GENTZ REINDER L/IN
E5           45     GENTZ REINER/IN
E6           95     GENTZ REINER L/IN
E7            1     GENTZ ROBERT E/IN
E8            1     GENTZ SOLANGE H/IN
E9            3     GENTZ SOLANGE H L/IN
E10           1     GENTZEL FREDERICK F/IN
E11           1     GENTZEL JR ALFRED F/IN
E12           2     GENTZHORN MICHAEL D/IN
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=> s e3-e7
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             1 "GENTZ REINDER L"/IN
            45 "GENTZ REINER"/IN
            95 "GENTZ REINER L"/IN
             1 "GENTZ ROBERT E"/IN
L31        142 ("GENTZ R"/IN OR "GENTZ REINDER L"/IN OR "GENTZ REINER"/IN OR
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         19692 ANTIVIRAL?
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          9476 KILLER
        183479 ENHANCING
        793115 FACTOR?
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                 (NATURAL(W)KILLER(W)ENHANCING(W)FACTOR?)
            59 PEROXIREDOXIN?
L32         56 L31 AND (NKEF? OR ANTIVIRAL? OR NATURAL KILLER ENHANCING FACTOR?
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=> s l32 and (antivir?)
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L33         56 L32 AND (ANTIVIR?)

=> s l32 and (antivir?)
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L33         56 L32 AND (ANTIVIR?)

=> d his

     (FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

     FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
                E LYNN RALF GEIBEN/IN
L1            1 S E3
                E WALKER BRUCE D/IN
L2            7 S E3
L3            6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
                E LYNN R G/IN
L4            2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
                E LYNN R G/AU
                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5            5 S E3 OR E4
                E WALKER B D/AU
L6          155 S E3
L7          154 S L6 NOT L5
L8            0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9          127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10         101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
L12         526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13         120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
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L14          125 S L13 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHA
L15           11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16           10 S L15 NOT (L1 OR L11)
L17          109 S L13 NOT L15
L18           54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19          535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20            3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21            3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22            2 S L21 NOT L20
                 E BUTTERFIELD L H/AU
L23           23 S E3
                 E SHAU H/AU
L24           65 S E3-E5
L25            1 S L19 AND (ANTIVIR?)

     FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
L26          105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L27            3 S L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR
L28            2 S L27 NOT L4

     FILE 'IFIPAT' ENTERED AT 12:59:18 ON 02 MAY 2004
L29            3 S (US 2001-952843)/PN,APPS AND AB/FA

     FILE 'USPATFULL' ENTERED AT 12:59:18 ON 02 MAY 2004
L30            3 S (US 2001-952843)/PN,APPS

     FILE 'USPATFULL' ENTERED AT 12:59:38 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 13:01:29 ON 02 MAY 2004
                 E GENTZ R/IN
L31          142 S E3-E7
L32           56 S L31 AND (NKEF? OR ANTIVIRAL? OR NATURAL KILLER ENHANCING FACT
L33           56 S L32 AND (ANTIVIR?)

=> d l33,ti,1-56

L33  ANSWER 1 OF 56  USPATFULL on STN
TI     Death domain containing receptors

L33  ANSWER 2 OF 56  USPATFULL on STN
TI     Keratinocyte growth factor-2

L33  ANSWER 3 OF 56  USPATFULL on STN
TI     Tumor necrosis factor receptors 6 alpha & 6 beta

L33  ANSWER 4 OF 56  USPATFULL on STN
TI     28 human secreted proteins

L33  ANSWER 5 OF 56  USPATFULL on STN
TI     Galectin 11

L33  ANSWER 6 OF 56  USPATFULL on STN
TI     T1 receptor-like ligand II and uses thereof

L33  ANSWER 7 OF 56  USPATFULL on STN
TI     Keratinocyte growth factor-2

L33  ANSWER 8 OF 56  USPATFULL on STN
TI     Synferon

L33  ANSWER 9 OF 56  USPATFULL on STN
TI     Death domain containing receptors

L33  ANSWER 10 OF 56  USPATFULL on STN
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TI Human tumor necrosis factor delta and epsilon

L33 ANSWER 11 OF 56 USPATFULL on STN
TI Leukocyte regulatory factors 1 and 2

L33 ANSWER 12 OF 56 USPATFULL on STN
TI Galectin-11 polypeptides

L33 ANSWER 13 OF 56 USPATFULL on STN
TI Keratinocyte growth factor-2

L33 ANSWER 14 OF 56 USPATFULL on STN
TI Antibodies to tumor necrosis factor 5

L33 ANSWER 15 OF 56 USPATFULL on STN
TI Human cystatin F

L33 ANSWER 16 OF 56 USPATFULL on STN
TI Methods of treating or preventing cell, tissue, and organ damage using human myeloid progenitor inhibitory factor-1 (MPIF-1)

L33 ANSWER 17 OF 56 USPATFULL on STN
TI Death domain containing receptor 4

L33 ANSWER 18 OF 56 USPATFULL on STN
TI Human cystatin F antibodies

L33 ANSWER 19 OF 56 USPATFULL on STN
TI KERATINOCYTE GROWTH FACTOR-2

L33 ANSWER 20 OF 56 USPATFULL on STN
TI Synferon

L33 ANSWER 21 OF 56 USPATFULL on STN
TI Polynucleotides encoding human tumor necrosis factor delta

L33 ANSWER 22 OF 56 USPATFULL on STN
TI Human polynucleotides, polypeptides, and antibodies

L33 ANSWER 23 OF 56 USPATFULL on STN
TI 28 human secreted proteins

L33 ANSWER 24 OF 56 USPATFULL on STN
TI Human tumor necrosis factor receptor TR9

L33 ANSWER 25 OF 56 USPATFULL on STN
TI Method and system for providing real-time, in situ biomanufacturing process monitoring and control in response to IR spectroscopy

L33 ANSWER 26 OF 56 USPATFULL on STN
TI Tumor necrosis factor receptors 6alpha & 6beta

L33 ANSWER 27 OF 56 USPATFULL on STN
TI T1 Receptor-like ligand II and uses thereof

L33 ANSWER 28 OF 56 USPATFULL on STN
TI Tumor necrosis factor receptor 5

L33 ANSWER 29 OF 56 USPATFULL on STN
TI Pancreas-derived plasminogen activator inhibitor

L33 ANSWER 30 OF 56 USPATFULL on STN
TI Death domain containing receptor-4

L33 ANSWER 31 OF 56 USPATFULL on STN
TI Human tumor necrosis factor delta and epsilon

L33 ANSWER 32 OF 56 USPATFULL on STN
TI Natural killer cell enhancing factor C

L33 ANSWER 33 OF 56 USPATFULL on STN
TI 28 human secreted proteins

L33 ANSWER 34 OF 56 USPATFULL on STN
TI 28 human secreted proteins

L33 ANSWER 35 OF 56 USPATFULL on STN
TI Death domain containing receptor 5

L33 ANSWER 36 OF 56 USPATFULL on STN
TI Human tumor necrosis factor delta and epsilon

L33 ANSWER 37 OF 56 USPATFULL on STN
TI Method and system for providing real-time, in situ biomanufacturing process monitoring and control in response to IR spectroscopy

L33 ANSWER 38 OF 56 USPATFULL on STN
TI Human cystatin E

L33 ANSWER 39 OF 56 USPATFULL on STN
TI Expression control sequences

L33 ANSWER 40 OF 56 USPATFULL on STN
TI Antibodies to human tumor necrosis factor receptor TR9

L33 ANSWER 41 OF 56 USPATFULL on STN
TI Pancreas-derived plasminogen activator inhibitor

L33 ANSWER 42 OF 56 USPATFULL on STN
TI Antibodies to human cystatin E

L33 ANSWER 43 OF 56 USPATFULL on STN
TI Natural killer cell enhancing factor C

L33 ANSWER 44 OF 56 USPATFULL on STN
TI Polynucleotides encoding natural killer cell enhancing factor C

L33 ANSWER 45 OF 56 USPATFULL on STN
TI Expression control sequences

L33 ANSWER 46 OF 56 USPATFULL on STN
TI Synferon, a synthetic interferon

L33 ANSWER 47 OF 56 USPATFULL on STN
TI Keratinocyte growth factor-2

L33 ANSWER 48 OF 56 USPATFULL on STN
TI Human cystatin F

L33 ANSWER 49 OF 56 USPATFULL on STN
TI Human cystatin E

L33 ANSWER 50 OF 56 USPATFULL on STN
TI Polynucleotides encoding natural killer cell enhancing factor C

L33 ANSWER 51 OF 56 USPATFULL on STN
TI DNA encoding human cystatin E

L33 ANSWER 52 OF 56 USPATFULL on STN
TI Ubiquitin conjugating enzymes 8 and 9

L33 ANSWER 53 OF 56 USPATFULL on STN

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L1     Ubiquitin conjugating enzymes 7, 8 and 9

L33  ANSWER 54 OF 56  USPATFULL on STN
TI     Human cystatin F

L33  ANSWER 55 OF 56  USPATFULL on STN
TI     Ubiquitin conjugating enzymes 7,8 and 9

L33  ANSWER 56 OF 56  USPATFULL on STN
TI     Ubiquitin conjugating enzymes 8 and 9


=> d his

     (FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

     FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
                E LYNN RALF GEIBEN/IN
L1              1 S E3
                E WALKER BRUCE D/IN
L2              7 S E3
L3              6 S L2 NOT L1

     FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
                E LYNN R G/IN
L4              2 S E3

     FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
                E LYNN R G/AU
                E LYNN RALF G/AU
                E GEIBEN-LYNN R/AU
                E GEIBEN LYNN R/AU
L5              5 S E3 OR E4
                E WALKER B D/AU
L6            155 S E3
L7            154 S L6 NOT L5
L8              0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9            127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10           101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11             1 S E2
L12           526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13           120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14           120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15            11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16            10 S L15 NOT (L1 OR L11)
L17           109 S L13 NOT L15
L18            54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19           535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20             3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21             3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22             2 S L21 NOT L20
                E BUTTERFIELD L H/AU
L23            23 S E3
                E SHAU H/AU
L24            65 S E3-E5
L25             1 S L19 AND (ANTIVIR?)

     FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
L26           105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
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L27 [illegible]
L28 2 S L27 NOT L4

FILE 'IFIPAT' ENTERED AT 12:59:18 ON 02 MAY 2004
L29 3 S (US 2001-952843)/PN,APPS AND AB/FA

FILE 'USPATFULL' ENTERED AT 12:59:18 ON 02 MAY 2004
L30 3 S (US 2001-952843)/PN,APPS

FILE 'USPATFULL' ENTERED AT 12:59:38 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 13:01:29 ON 02 MAY 2004
E GENTZ R/IN
L31 142 S E3-E7
L32 56 S L31 AND (NKEF? OR ANTIVIRAL? OR NATURAL KILLER ENHANCING FACT
L33 56 S L32 AND (ANTIVIR?)

=> s l33 and ay<2002
3448540 AY<2002
L34 39 L33 AND AY<2002

=> d l34,cbib,ab,1-39

L34 ANSWER 1 OF 39 USPATFULL on STN
2004:78840 Death domain containing receptors.
Yu, Guo-Liang, Berkeley, CA, United States
Ni, Jian, Rockville, MD, United States
Dixit, Vishva M., Los Altos Hills, CA, United States
Gentz, Reiner L., Rockville, MD, United States
Dillon, Patrick J., Carlsbad, CA, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6713061 B1 20040330
APPLICATION: US 2000-557908 20000421 (9) <--
PRIORITY: US 1999-136741P 19990528 (60)
US 1999-130488P 19990422 (60)
US 1997-37341P 19970206 (60)
US 1996-28711P 19961017 (60)
US 1996-13285P 19960312 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel Death Domain Containing Receptor (DR3 and DR3-V1) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR3 and DR3-V1 proteins. DR3 and DR3-V1 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR3 and DR3-V1 activity.

L34 ANSWER 2 OF 39 USPATFULL on STN
2004:41451 Keratinocyte growth factor-2.
Ruben, Steven M., Brookeville, MD, United States
Jimenez, Pablo, Chatham, NJ, United States
Duan, D. Roxanne, Gaithersburg, MD, United States
Rampy, Mark A., Montgomery Village, MD, United States
Mendrick, Donna, Mount Airy, MD, United States
Zhang, Jun, San Diego, CA, United States
NI, Jian, Germantown, MD, United States
Moore, Paul A., North Bethesda, MD, United States
Coleman, Timothy A., Gaithersburg, MD, United States
Gruber, Joachim R., Dallas, TX, United States
Dillon, Patrick J., Carlsbad, CA, United States
Gentz, Reiner L., Belo Horizonte-Mg, BRAZIL
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

APPLICATION: US 2000-610651 20000630 (9) <--
PRIORITY: US 2000-205417P 20000519 (60)
US 2000-198322P 20000419 (60)
US 1999-171677P 19991222 (60)
US 1999-163375P 19991103 (60)
US 1999-149935P 19990819 (60)
US 1999-148628P 19990812 (60)
US 1999-144024P 19990715 (60)
US 1999-143648P 19990714 (60)
US 1999-142343P 19990702 (60)
US 1997-39045P 19970228 (60)
US 1997-55561P 19970813 (60)
US 1996-23852P 19960813 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a Keratinocyte Growth Factor, sometimes hereinafter referred to as "KGF-2" also formerly known as Fibroblast Growth Factor 12 (FGF-12). This invention further relates to the therapeutic use of KGF-2 to promote or accelerate wound healing. This invention also relates to novel mutant forms of KGF-2 that show enhanced activity, increased stability, higher yield or better solubility.

L34 ANSWER 3 OF 39 USPATFULL on STN
2003:216222 Galectin-11 polypeptides.
Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Rockville, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Liu, Fu-Tong, San Diego, CA, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)La Jolla Institute for Allergy and Immunology, San Diego, CA, United States (U.S. corporation)
US 6605699 B1 20030812
APPLICATION: US 2000-557170 20000421 (9) <--
PRIORITY: US 1997-34205P 19970121 (60)
US 1997-34204P 19970121 (60)
US 1999-169932P 19991210 (60)
US 1999-130390P 19990421 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to galectin 11 proteins which are members of the galectin superfamily. In particular, the present invention relates to full-length polypeptides, fragments, and variants of galectin 11.

L34 ANSWER 4 OF 39 USPATFULL on STN
2003:155715 Human cystatin F antibodies.
Li, Haodong, Gaitheresburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Ni, Jian, Rockville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6576745 B1 20030610
APPLICATION: US 2000-528436 20000317 (9) <--
PRIORITY: US 1996-14795P 19960403 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to Cystatin F polypeptides, polynucleotides encoding the polypeptides, methods for producing the polypeptides, in particular by expressing the polynucleotides, and agonists and antagonists of the polypeptides. The invention further relates to

methods for detecting such polynucleotides, polypeptides, agonists and antagonists for applications, which relate, in part, to research, diagnostic and clinical arts.

L34 ANSWER 5 OF 39 USPATFULL on STN
2003:112962 KERATINOCYTE GROWTH FACTOR-2.
RUBEN, STEVEN M., OLNEY, MD, UNITED STATES
JIMENEZ, PABLO, ELLICOTT CITY, MD, UNITED STATES
DUAN, D. ROXANNE, BETHESDA, MD, UNITED STATES
RAMPY, MARK A., GAITHERSBURG, MD, UNITED STATES
MENDRICK, DONNA, MT. AIRY, MD, UNITED STATES
ZHANG, JUN, BETHESDA, MD, UNITED STATES
NI, JIAN, ROCKVILLE, MD, UNITED STATES
MOORE, PAUL A., GERMANTOWN, MD, UNITED STATES
COLEMAN, TIMOTHY A., GAITHERSBURG, MD, UNITED STATES
GRUBER, JOACHIM R., CHESTNUT HILL, MA, UNITED STATES
DILLON, PATRICK J., CARLSBAD, CA, UNITED STATES
GENTZ, REINER L., ROCKVILLE, MD, UNITED STATES
HUMAN GENOME SCIENCES, INC. (U.S. corporation)
US 2003077695 A1 20030424
APPLICATION: US 1999-345373 A1 19990701 (9) <--
PRIORITY: US 1997-39405P 19970228 (60)
US 1997-55561P 19970813 (60)
US 1996-23852P 19960813 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a Keratinocyte Growth Factor, sometimes hereinafter referred to as "KGF-2" also formerly known as Fibroblast Growth Factor 12 (FGF-12). This invention further relates to the therapeutic use of KGF-2 to promote or accelerate wound healing. This invention also relates to novel mutant forms of KGF-2 that show enhanced activity, increased stability, higher yield or better solubility.

L34 ANSWER 6 OF 39 USPATFULL on STN
2003:102136 Synferon.
Olsen, Henrik S., Gaithersburg, MD, United States
Gentz, Reiner L., Rockville, MD, United States
Ruben, Steven M., Olney, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6548063 B1 20030415
APPLICATION: US 2000-604073 20000627 (9) <--
PRIORITY: US 1997-67746P 19971205 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a novel Synferon protein which is a member of the interferon family. In particular, isolated nucleic acid molecules are provided encoding a synthetic interferon polypeptide, called "Synferon". Synferon polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of Synferon activity. Also provided are therapeutic methods for treating immune system-related disorders.

L34 ANSWER 7 OF 39 USPATFULL on STN
2003:20123 Polynucleotides encoding human tumor necrosis factor delta.
Yu, Guo-Liang, Darnestown, MD, United States
Ni, Jian, Rockville, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Dillon, Patrick J., Gaithersburg, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

APPLICATION: US 1997-815783 19970312 (8) <--
PRIORITY: US 1996-16812P 19960314 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to human TNF delta and TNF epsilon polypeptides, polynucleotides encoding the polypeptides, methods for producing the polypeptides, in particular by expressing the polynucleotides, and agonists and antagonists of the polypeptides. The invention further relates to methods for utilizing such polynucleotides, polypeptides, agonists and antagonists for applications, which relate, in part, to research, diagnostic and clinical arts.

L34 ANSWER 8 OF 39 USPATFULL on STN
2002:337390 Human polynucleotides, polypeptides, and antibodies.
Moore, Paul A., Germantown, MD, UNITED STATES
Coleman, Timothy A., Gaithersburg, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Dillon, Patrick J., Carlsbad, CA, UNITED STATES
Ni, Jian, Germantown, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES
Endress, Gregory A., Florence, MA, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
US 2002192749 A1 20021219
APPLICATION: US 2001-969384 A1 20011003 (9) <--
PRIORITY: US 2000-194118P 20000403 (60)
US 2000-236384P 20000929 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel human human polypeptides and isolated nucleic acids containing the coding regions of the genes encoding such polypeptides. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human human polypeptides. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human human polypeptides.

L34 ANSWER 9 OF 39 USPATFULL on STN
2002:307870 28 human secreted proteins.
Ruben, Steven M., Olney, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES
Zeng, Zhizhen, Lansdale, PA, UNITED STATES
Kyaw, Hla, Frederick, MD, UNITED STATES
Fischer, Carrie L., Burke, VA, UNITED STATES
Li, Haodong, Gaithersburg, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Wei, Ying-Fei, Berkeley, CA, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Young, Paul E., Gaithersburg, MD, UNITED STATES
Greene, John M., Gaithersburg, MD, UNITED STATES
Ferrie, Ann M., Tewksbury, MA, UNITED STATES
US 2002172994 A1 20021121
APPLICATION: US 2001-852797 A1 20010511 (9) <--
PRIORITY: US 2001-265583P 20010202 (60)
US 1997-40762P 19970314 (60)
US 1997-40710P 19970314 (60)
US 1997-50934P 19970530 (60)
US 1997-48100P 19970530 (60)
US 1997-48357P 19970530 (60)
US 1997-48189P 19970530 (60)
US 1997-57765P 19970905 (60)
US 1997-48970P 19970606 (60)
US 1997-68368P 19971219 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

L34 ANSWER 10 OF 39 USPATFULL on STN

2002:272468 Tumor necrosis factor receptors 6alpha & 6beta.

Gentz, Reiner L., Rockville, MD, UNITED STATES
Ebner, Reinhard, Gaithersburg, MD, UNITED STATES
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES
Ni, Jian, Germantown, MD, UNITED STATES
Feng, Ping, Gaithersburg, MD, UNITED STATES
Human Genome Sciences, Inc., Rockville, MD, UNITED STATES, 20850 (U.S. corporation)

US 2002150583 A1 20021017
APPLICATION: US 2001-935727 A1 20010824 (9) <--
PRIORITY: US 2001-303224P 20010706 (60)
US 2000-252131P 20001121 (60)
US 2000-227598P 20000825 (60)
US 1999-168235P 19991201 (60)
US 1999-146371P 19990802 (60)
US 1999-131964P 19990430 (60)
US 1999-131270P 19990427 (60)
US 1999-124092P 19990312 (60)
US 1999-121774P 19990304 (60)
US 1997-35496P 19970114 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel Tumor Necrosis Factor Receptor proteins. In particular, isolated nucleic acid molecules are provided encoding the human TNFR-6α & -6β proteins. TNFR-6α & -6β polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of TNFR-6α & -6β activity. Also provided are diagnostic methods for detecting immune system-related disorders and therapeutic methods for treating immune system-related disorders.

L34 ANSWER 11 OF 39 USPATFULL on STN

2002:258891 T1 Receptor-like ligand II and uses thereof.

Ni, Jian, Rockville, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES

US 2002142461 A1 20021003
APPLICATION: US 2000-731924 A1 20001208 (9) <--
PRIORITY: US 1996-24348P 19960823 (60)
US 1999-169979P 19991210 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a novel T1 Receptor (T1R)-like ligand II protein. In particular, isolated nucleic acid molecules are provided encoding the T1R-like ligand II protein. T1R-like ligand II polypeptides are also provided, as are recombinant vectors and host cells for expressing the same. This invention further relates to pharmaceutical compositions and formulations comprising T1R-like ligand II. Also provided are methods of using T1R-like ligand II polynucleotides, polypeptides, antibodies or agonists/antagonists for therapeutic and diagnostic purposes. Diagnostic kits are further provided.

L34 ANSWER 12 OF 39 USPATFULL on STN

2002:246365 Tumor necrosis factor receptor 5.

Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Rockville, MD, United States
Ruben, Steven M., Odenton, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6455040 B1 20020924
APPLICATION: US 2000-573986 20000518 (9) <--
PRIORITY: US 1999-135164P 19990520 (60)
US 1997-54885P 19970807 (60)
US 1997-35496P 19970114 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a novel human gene encoding a polypeptide which is a member of the TNF receptor family, and has now been found to bind TRAIL. More specifically, an isolated nucleic acid molecule is provided encoding a human polypeptide named tumor necrosis factor receptor-5, sometimes referred to as "TNFR-5" or "TR5," and now referred to hereinafter as "TRAIL receptor without intracellular domain" or "TRID." TRID polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same as well as anti-TRID antibodies. The invention further relates to screening methods for identifying agonists or antagonists of TRAIL polypeptide activity. Also provided are diagnostic and therapeutic methods utilizing such compositions.

L34 ANSWER 13 OF 39 USPATFULL on STN
2002:235451 Pancreas-derived plasminogen activator inhibitor.
Ni, Jian, Rockville, MD, UNITED STATES
Gentz, Reiner L., Silver Spring, MD, UNITED STATES
Ruben, Steven M., Olney, MD, UNITED STATES
Shi, Y. Eric, Roslyn Heights, NY, UNITED STATES
Human Genome Sciences, Inc. (U.S. corporation)
US 2002127640 A1 20020912
APPLICATION: US 2001-902684 A1 20010712 (9) <--
PRIORITY: US 1996-24056P 19960816 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a novel member of the plasminogen activator inhibitor protein family. In particular, isolated nucleic acid molecules are provided encoding the pancreas-derived plasminogen activator inhibitor protein. Pancreas-derived plasminogen activator inhibitor polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to methods for treating physiologic and pathologic disease conditions, including breast cancer, and diagnostic methods for detecting pathologic disorders.

L34 ANSWER 14 OF 39 USPATFULL on STN
2002:202241 Death domain containing receptor-4.
Ni, Jian, Rockville, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Pan, James G., Belmont, CA, United States
Gentz, Reiner L., Rockville, MD, United States
Dixit, Vishva M., Los Altos Hills, CA, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)The Regents of the University of Michigan, Ann Arbor, MI, United States (U.S. corporation)
US 6433147 B1 20020813
APPLICATION: US 2000-565918 20000505 (9) <--
PRIORITY: US 1999-132922P 19990506 (60)
US 1997-35722P 19970128 (60)
US 1997-37829P 19970205 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel Death Domain Containing

receptor 4 (DR4) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR4 proteins. DR4 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR4 activity and methods for using DR4 polynucleotides and polypeptides.

L34 ANSWER 15 OF 39 USPATFULL on STN
2002:198230 Natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, UNITED STATES
Yu, Guo-Liang, Darnestown, MD, UNITED STATES
Gentz, Reiner, Silver Spring, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
US 2002106323 A1 20020808
APPLICATION: US 2001-911346 A1 20010724 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and fragments thereof and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptidees by recombinant techniques is disclosed. Further disclosed are antibodies directed against such polypeptides and fragments or portions thereof and methods for producing such antibodies and utilizing such antibodies for therapeutic or diagnostic purposes. Also disclosed are methods for utilizing such polypeptides and/or antibodies for preventing and/or treating viral infections, inflammation, neoplasia and damge from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

L34 ANSWER 16 OF 39 USPATFULL on STN
2002:149131 28 human secreted proteins.
Ruben, Steven M., Olney, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES
Zeng, Zhizhen, Lansdale, PA, UNITED STATES
Kyaw, Hla, Frederick, MD, UNITED STATES
Fischer, Carrie L., Burke, VA, UNITED STATES
Li, Haodong, Gaithersburg, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Wei, Ying-Fei, Berkeley, CA, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Young, Paul E., Gaithersburg, MD, UNITED STATES
Greene, John M., Gaithersburg, MD, UNITED STATES
Ferrie, Ann M., Tewksbury, MA, UNITED STATES
US 2002077287 A1 20020620
APPLICATION: US 2001-852659 A1 20010511 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

L34 ANSWER 17 OF 39 USPATFULL on STN
2002:148614 28 human secreted proteins.
Ruben, Steven M., Olney, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Li, Yi, Sunnyvale, CA, UNITED STATES
Zeng, ZhiZhen, Lansdale, PA, UNITED STATES

Fischer, Carrie L., Burke, VA, UNITED STATES
Li, Haodong, Gaithersburg, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Wei, Ying-Fei, Berkeley, CA, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Young, Paul E., Gaithersburg, MD, UNITED STATES
Greene, John M., Gaithersburg, MD, UNITED STATES
Ferrie, Ann M., Painted Post, NY, UNITED STATES
US 2002076756 A1 20020620
APPLICATION: US 2001-853161 A1 20010511 (9) <--
PRIORITY: US 2001-265583P 20010202 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

L34 ANSWER 18 OF 39 USPATFULL on STN
2002:141109 Death domain containing receptor 5.
Ni, Jian, Rockville, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Rosen, Craig A., Laytonville, MD, UNITED STATES
Human Genome Sciences, Inc., Rockville, MD, 20850 (U.S. corporation)
US 2002072091 A1 20020613
APPLICATION: US 2001-874138 A1 20010606 (9) <--
PRIORITY: US 1999-148939P 19990813 (60)
US 1999-133238P 19990507 (60)
US 1999-132498P 19990504 (60)
US 1997-40846P 19970317 (60)
US 1997-54021P 19970729 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to novel Death Domain Containing Receptor-5 (DR5) proteins which are members of the tumor necrosis factor (TNF) receptor family, and have now been shown to bind TRAIL. In particular, isolated nucleic acid molecules are provided encoding the human DR5 proteins. DR5 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying antagonists and antagonists of DR5 activity.

L34 ANSWER 19 OF 39 USPATFULL on STN
2002:126317 Human tumor necrosis factor delta and epsilon.
Yu, Guo-Liang, Berkeley, CA, UNITED STATES
Ni, Jian, Germantown, MD, UNITED STATES
Gentz, Reiner L., Rockville, MD, UNITED STATES
Dillon, Patrick J., Carlsbad, CA, UNITED STATES
Human Genome Sciences, Inc., Rockville, MD, UNITED STATES, 20850 (U.S. corporation)
US 2002064829 A1 20020530
APPLICATION: US 2001-879919 A1 20010614 (9) <--
PRIORITY: US 1996-16812P 19960314 (60)
US 2001-293499P 20010525 (60)
US 2001-277978P 20010323 (60)
US 2001-276248P 20010316 (60)
US 2000-254875P 20001213 (60)
US 2000-241952P 20001023 (60)
US 2000-211537P 20000615 (60)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to human TNF delta and TNF epsilon polypeptides, polynucleotides encoding the polypeptides, methods for producing the polypeptides, in particular by expressing the polynucleotides, and agonists and antagonists of the polypeptides. The invention further relates to methods for utilizing such polynucleotides, polypeptides, agonists and antagonists for applications, which relate, in part, to research, diagnostic and clinical arts.

L34 ANSWER 20 OF 39 USPATFULL on STN

2002:122481 Method and system for providing real-time, in situ biomanufacturing process monitoring and control in response to IR spectroscopy.

Naughton, Raymond A., West River, MD, United States

Rohrer, Thomas R., Hagerstown, MD, United States

Gentz, Reiner L., Rockville, MD, United States

Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

US 6395538 B1 20020528

APPLICATION: US 2000-616894 20000714 (9) <--

PRIORITY: US 1999-157863P 19991006 (60)

US 1999-144071P 19990716 (60)

US 1999-151918P 19990901 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method and system for providing real-time, biomanufacturing process monitoring and control in response to infra-red (IR) spectroscopic fingerprinting of a biomolecule. IR spectroscopy is used to fingerprint an active biomolecule in situ in a biomanufacturing process. In one embodiment, Fourier Transform Infra-red spectroscopy (FTIR) is used to determine whether an active or aged biomolecule is present in stages of a biomanufacturing process. In one preferred example, the biomanufacturing process manufactures a biomaterial in bulk. The biomanufacturing process has four stages: bioproduction, recovery, purification, and bulk storage. FTIR spectroscopy is used to monitor the optimization of each process step by providing feedback controls, and to fingerprint in real-time, in situ whether active biomolecules are present in each stage.

L34 ANSWER 21 OF 39 USPATFULL on STN

2002:99575 Human cystatin E.

Ni, Jian, Germantown, MD, UNITED STATES

Gentz, Reiner L., Rockville, MD, UNITED STATES

Yu, Guo-Liang, Berkeley, CA, UNITED STATES

Rosen, Craig A., Laytonsville, MD, UNITED STATES

Human Genome Sciences, Inc., Rockville, MD, UNITED STATES, 20850 (U.S. corporation)

US 2002052476 A1 20020502

APPLICATION: US 2001-940497 A1 20010829 (9) <--

DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a human CysE polypeptide and DNA (RNA) encoding such polypeptide. Also provided is a procedure for producing such polypeptide by recombinant techniques. Also disclosed are methods for utilizing such polypeptide for treating osteoporosis, tumor metastases, microbial infections, viral infection, septic shock, inflammation, retinal irritation, caries, cachicia and muscle wasting. Diagnostic methods for detecting mutations in the coding sequence and alterations in the concentration of the polypeptides in a sample derived from a host are also disclosed.

L34 ANSWER 22 OF 39 USPATFULL on STN

2002:66934 Expression control sequences.

Gentz, Reiner L., Silver Spring, MD, UNITED STATES

Coleman, Timothy A., Gaithersburg, MD, UNITED STATES

US 2002037587 A1 20020328

APPLICATION: US 2000-725460 A1 20001130 (9) <--

DOCUMENT TYPE: Utility; APPLICATION:

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Expression control sequences are provided for the expression of proteins from a gene of interest. The gene may express viral, prokaryotic, or eukaryotic proteins. These control sequences are produced by combining phage promoter and operator/repressor systems. Expression vectors containing such expression control sequences, microorganisms transformed with such expression vectors and methods for producing viral, prokaryotic, and eukaryotic proteins using the expression control sequences, expression vectors and transformed microorganisms are also provided.

L34 ANSWER 23 OF 39 USPATFULL on STN

2002:57390 Antibodies to human tumor necrosis factor receptor TR9.

Ni, Jian, Rockville, MD, United States
Yu, Guo-Liang, Berkeley, CA, United States
Fan, Ping, Gaithersburg, MD, United States
Gentz, Reiner L., Rockville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6358508 B1 20020319
APPLICATION: US 2000-527236 20000316 (9) <--
PRIORITY: US 1997-52991P 19970611 (60)
US 1999-126019P 19990324 (60)
US 1999-134220P 19990514 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a novel member of the tumor necrosis factor family of receptors. In particular, isolated nucleic acid molecules are provided encoding the human TR9 receptor. TR9 polypeptides are also provided as are antibodies vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of TR9 receptor activity.

L34 ANSWER 24 OF 39 USPATFULL on STN

2001:178842 Pancreas-derived plasminogen activator inhibitor.

Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Ruben, Steven M., Olney, MD, United States
Shi, Y. Eric, Roslyn Heights, NY, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6303338 B1 20011016
APPLICATION: US 1998-26408 19980219 (9) <--
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a novel member of the plasminogen activator inhibitor protein family. In particular, isolated nucleic acid molecules are provided encoding the pancreas-derived plasminogen activator inhibitor protein. Pancreas-derived plasminogen activator inhibitor polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to methods for treating physiologic and pathologic disease conditions, including breast cancer, and diagnostic methods for detecting pathologic disorders.

L34 ANSWER 25 OF 39 USPATFULL on STN

2001:173721 Antibodies to human cystatin E.

Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6300477 B1 20011009

APPLICATION: US 1999-[illegible] 19990[illegible] (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are antibodies and antibody fragments that bind to novel human CysE polypeptides. Also provided are methods for producing the antibodies. Also disclosed are methods for utilizing such antibodies and antibody fragments for the purification and analysis of CysE polypeptides, as well as the diagnosis and treatment of CysE polypeptide associated diseases.

L34 ANSWER 26 OF 39 USPATFULL on STN
2001:162839 Natural killer cell enhancing factor C.
Ni, Jiar, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6294164 B1 20010925
APPLICATION: US 1999-407891 19990929 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

L34 ANSWER 27 OF 39 USPATFULL on STN
2001:102589 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6255079 B1 20010703
APPLICATION: US 1995-467265 19950606 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

L34 ANSWER 28 OF 39 USPATFULL on STN
2001:29321 Expression control sequences.
Gentz, Reiner L., Silver Spring, MD, United States
Coleman, Timothy A., Gaithersburg, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6194168 B1 20010227
APPLICATION: US 1998-44796 19980320 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Expression control sequences are provided for the expression of proteins from a gene of interest. The gene may express viral, prokaryotic, or

eukaryotic promoter. These control sequences are produced by combining phage promoter and operator/repressor systems. Expression vectors containing such expression control sequences, microorganisms transformed with such expression vectors and methods for producing viral, prokaryotic, and eukaryotic proteins using the expression control sequences, expression vectors and transformed microorganisms are also provided.

L34 ANSWER 29 OF 39 USPATFULL on STN
2000:117526 Synferon, a synthetic interferon.
Olsen, Henrik S., Gaithersburg, MD, United States
Gentz, Reiner L., Rockville, MD, United States
Ruben, Steven M., Olney, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6114145 20000905
APPLICATION: US 1998-205264 19981202 (9) <--
PRIORITY: US 1997-67746P 19971205 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a novel Synferon protein which is a member of the interferon family. In particular, isolated nucleic acid molecules are provided encoding a synthetic interferon polypeptide, called "Synferon". Synferon polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of Synferon activity. Also provided are therapeutic methods for treating immune system-related disorders.

L34 ANSWER 30 OF 39 USPATFULL on STN
2000:77208 Keratinocyte growth factor-2.
Ruben, Steven M., Olney, MD, United States
Jimenez, Pablo, Ellicott City, MD, United States
Duan, D. Roxanne, Bethesda, MD, United States
Rampy, Mark A., Gaithersburg, MD, United States
Mendrick, Donna, Mt. Airy, MD, United States
Zhang, Jun, Bethesda, MD, United States
Ni, Jian, Rockville, MD, United States
Moore, Paul A., Germantown, MD, United States
Coleman, Timothy A., Gaithersburg, MD, United States
Gruber, Joachim R., Chestnut Hill, MA, United States
Dillon, Patrick J., Carlsbad, CA, United States
Gentz, Reiner L., Rockville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6077692 20000620
APPLICATION: US 1998-23082 19980213 (9) <--
PRIORITY: US 1997-55561P 19970813 (60)
US 1997-39045P 19970228 (60)
US 1996-23852P 19960813 (60)
US 1997-68493P 19971222 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is a Keratinocyte Growth Factor, sometimes hereinafter referred to as "KGF-2" also formerly known as Fibroblast Growth Factor 12 (FGF-12). This invention further relates to the therapeutic use of KGF-2 to promote or accelerate wound healing. This invention also relates to novel mutant forms of KGF-2 that show enhanced activity, increased stability, higher yield or better solubility.

L34 ANSWER 31 OF 39 USPATFULL on STN
2000:64843 Human cystatin F.

Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Ni, Jian, Rockville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6066617 20000523
APPLICATION: US 1998-19485 19980129 (9) <--
PRIORITY: US 1996-14795P 19960403 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to Cystatin F polypeptides, polynucleotides encoding the polypeptides, methods for producing the polypeptides, in particular by expressing the polynucleotides, and agonists and antagonists of the polypeptides. The invention further relates to methods for utilizing such polynucleotides, polypeptides, agonists and antagonists for applications, which relate, in part, to research, diagnostic and clinical arts.

L34 ANSWER 32 OF 39 USPATFULL on STN
2000:1855 Human cystatin E.
Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6011012 20000104
APPLICATION: US 1996-744138 19961105 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a human CysE polypeptide and DNA (RNA) encoding such polypeptide. Also provided is a procedure for producing such polypeptide by recombinant techniques. Also disclosed are methods for utilizing such polypeptide for treating osteoporosis, tumor metastases, microbial infections, viral infection, septic shock, inflammation, retinal irritation, caries, cachicia and muscle wasting. Diagnostic methods for detecting mutations in the coding sequence and alterations in the concentration of the polypeptides in a sample derived from a host are also disclosed.

L34 ANSWER 33 OF 39 USPATFULL on STN
1999:146312 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, 305 W. Side Dr., Apt. #204, Gaithersburg, MD, United States 20878
Yu, Guo-Liang, 13524 Straw Bale La., Darnestown, MD, United States 20878
Gentz, Reiner, 13404 Fairland Park Dr., Silver Spring, MD, United States 20904
Rosen, Craig A., 22400 Rolling Hill Rd., Laytonsville, MD, United States 20882
US 5985612 19991116
APPLICATION: US 1995-467265 19950606 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

L34 ANSWER 34 OF 39 USPATFULL on STN
1999:146301 DNA encoding human cystatin E.

Ni, Jian, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 5985601 19991116
APPLICATION: US 1995-461030 19950605 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a human CysE polypeptide and DNA (RNA) encoding such polypeptide. Also provided is a procedure for producing such polypeptide by recombinant techniques. Also disclosed are methods for utilizing such polypeptide for treating osteoporosis, tumor metastases, microbial infections, viral infection, septic shock, inflammation, retinal irritation, caries, cachexia and muscle wasting. Diagnostic methods for detecting mutations in the coding sequence and alterations in the concentration of the polypeptides in a sample derived from a host are also disclosed.

L34 ANSWER 35 OF 39 USPATFULL on STN
1999:128413 Ubiquitin conjugating enzymes 8 and 9.
Ni, Jian, Gaithersburg, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Adams, Mark D., North Potomac, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 5968797 19991019
APPLICATION: US 1997-903396 19970722 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human UCE 7, UCE 8 and UCE 9 polypeptides and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods of utilizing such polypeptide for the treatment of the proliferation of malignant cells. Antagonists against such polypeptides and their uses as a therapeutic to treat Alzheimer's disease, atrophying skeletal muscle, African swine fever virus and apoptotic cell death are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in UCE 7, 8 and 9 nucleic acid sequences and the concentration of polypeptides encoded by such sequences.

L34 ANSWER 36 OF 39 USPATFULL on STN
1999:102710 Ubiquitin conjugating enzymes 7, 8 and 9.
Ni, Jian, Rockville, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Adams, Mark D., North Potomac, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 5945321 19990831
WO 9623410 19960808
APPLICATION: US 1997-875272 19971002 (8) <--
WO 1995-US1250 19950131 19971002 PCT 371 date 19971002 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human UCE 7, UCE 8 and UCE 9 polypeptides and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods of utilizing such polypeptides for the treatment of the porliferation of malignant cells. Antagonists against such polypeptides and their uses as a therapeutic to treat Alzheimer's disease, atrophying skeletal muscle, African Swine Fever Virus and apoptotic cell death are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in UCE 7, 8 and 9 nucleic acid sequences and to the concentration of polypeptides encoded by such sequences.

1999:75515 Human cystatin F.
Ni, Jian, Rockville, MD, United States
Li, Haodong, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner L., Silver Spring, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 5919658 19990706
APPLICATION: US 1997-832535 19970403 (8) <--
PRIORITY: US 1996-14795P 19960403 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to cystatin F polypeptides, polynucleotides encoding the polypeptides, methods for producing the polypeptides, in particular by expressing the polynucleotides, and agonists and antagonists of the polypeptides. The invention further relates to methods for utilizing such polynucleotides, polypeptides, agonists and antagonists for applications, which relate, in part, to research, diagnostic and clinical arts.

L34 ANSWER 38 OF 39 USPATFULL on STN
1998:156910 Ubiquitin conjugating enzymes 7,8 and 9.
Ni, Jian, Gaithersburg, MD, United States
Gentz, Reiner, Silver Springs, MD, United States
Adams, Mark D., North Potomac, MD, United States
Human Genome Sciences, Inc., Gaithersburg, MA, United States (U.S. corporation)
US 5849286 19981215
APPLICATION: US 1995-464604 19950605 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human UCE 7, UCE 8 and UCE 9 polypeptides and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods of utilizing such polypeptide for the treatment of the proliferation of malignant cells. Antagonists against such polypeptides and their uses as a therapeutic to treat Alzheimer's disease, atrophying skeletal muscle, African swine fever virus and apoptotic cell death are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in UCE 7, 8 and 9 nucleic acid sequences and the concentration of polypeptides encoded by such sequences.

L34 ANSWER 39 OF 39 USPATFULL on STN
97:63915 Ubiquitin conjugating enzymes 8 and 9.
Ni, Jian, Gaithersburg, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Adams, Mark D., North Potomac, MD, United States
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APPLICATION: US 1995-464342 19950605 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human UCE 7, UCE 8 and UCE 9 polypeptides and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods of utilizing such polypeptide for the treatment of the proliferation of malignant cells. Antagonists against such polypeptides and their uses as a therapeutic to treat Alzheimer's disease, atrophying skeletal muscle, African swine fever virus and apoptotic cell death are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in UCE 7, 8 and 9 nucleic acid sequences and the concentration of polypeptides encoded by such sequences.

L34 ANSWER 15 OF 39 USPATFULL on STN
2002:198230 Natural killer cell enhancing factor C.
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US 2002106323 A1 20020808
APPLICATION: US 2001-911346 A1 20010724 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A human natural killer cell enhancing factor C and fragments thereof and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptidees by recombinant techniques is disclosed. Further disclosed are antibodies directed against such polypeptides and fragments or portions thereof and methods for producing such antibodies and utilizing such antibodies for therapeutic or diagnostic purposes. Also disclosed are methods for utilizing such polypeptides and/or antibodies for preventing and/or treating viral infections, inflammation, neoplasia and damge from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

CLM What is claimed is:

1. An isolated antibody or portion thereof that specifically binds to a protein whose sequence consists of amino acid residues +31 to +271 of SEQ ID NO:2.

2. The antibody or portion thereof of claim 1 wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

3. The antibody or portion thereof of claim 1 which is a monoclonal antibody.

4. The antibody or portion thereof of claim 1 which is a polyclonal antibody.

5. The antibody or portion thereof of claim 1 which is a chimeric antibody.

6. The antibody or portion thereof of claim 1 which is a single chain antibody.

7. The antibody or portion thereof of claim 1 which is a Fab fragment.

8. The antibody or portion thereof of claim 1 which is labeled.

9. The antibody of claim 8 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

10. A composition comprising the antibody or portion thereof of claim 1 and a carrier.

11. The composition of claim 10, wherein the antibody or portion thereof is a monoclonal antibody.

12. The composition of claim 10, wherein the antibody or portion thereof is a polyclonal antibody.

13. The composition of claim 10, wherein the antibody or portion thereof is a chimeric antibody.

14. The composition of claim 10, wherein the antibody or portion thereof

is a single chain antibody.

15. The composition of claim 10, wherein the antibody or portion thereof is a Fab fragment.

16. The composition of claim 10, wherein the antibody or portion thereof is labeled.

17. The composition of claim 16 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

18. An isolated cell that produces the antibody or portion thereof of claim 1.

19. A hybridoma that produces the antibody of claim 1.

20. A hybridoma that produces the antibody of claim 3.

21. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 1; and (b) detecting the **NKEF** C protein in the biological sample.

22. The method of claim 21 wherein the antibody is a monoclonal antibody.

23. The method of claim 21 wherein the antibody is a polyclonal antibody.

24. The method of claim 21 wherein the antibody is a chimeric antibody.

25. The method of claim 21 wherein the antibody is a single chain antibody.

26. The method of claim 21 wherein the antibody is a Fab fragment.

27. The method of claim 21 wherein the antibody is a labeled antibody.

28. The method of claim 27 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

29. An isolated antibody or portion thereof produced by immunizing an animal with a protein whose sequence comprises amino acid residues +31 to +271 of SEQ ID NO:2; wherein said antibody or portion thereof specifically binds to the amino acid sequence of SEQ ID NO:2.

30. An isolated antibody or portion thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose sequence consists of amino acid residues +1 to +271 of SEQ ID NO:2; (b) a protein whose sequence consists of at least 30 contiguous amino acid residues of SEQ ID NO:2; and (c) a protein whose sequence consists of at least 50 contiguous amino acid residues of SEQ ID NO:2.

31. The isolated antibody or portion thereof of claim 30, that specifically binds protein (a).

32. The isolated antibody or portion thereof of claim 30, that specifically binds protein (b).

33. The isolated antibody or portion thereof of claim 30, that specifically binds protein (c).

34. The isolated antibody or portion thereof of claim 30, wherein said protein specifically bound by said isolated antibody or portion thereof

is glycosylated.

35. The isolated antibody or portion thereof of claim 30 which is a monoclonal antibody.

36. The isolated antibody or portion thereof of claim 30 which is a polyclonal antibody.

37. The isolated antibody or portion thereof of claim 30, which is a chimeric antibody.

38. The isolated antibody or portion thereof of claim 30 which is a single chain antibody.

39. The isolated antibody or portion thereof of claim 30 which is a Fab fragment.

40. The antibody or portion thereof of claim 30 which is labeled.

41. The antibody of claim 40 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

42. A composition comprising the isolated antibody or portion thereof of claim 30 and a carrier.

43. The composition of claim 42, wherein the isolated antibody or portion thereof is a monoclonal antibody.

44. The composition of claim 42, wherein the isolated antibody or portion thereof is a polyclonal antibody.

45. The composition of claim 42, wherein the isolated antibody or portion thereof is a chimeric antibody.

46. The composition of claim 42, wherein the isolated antibody or portion thereof is a single chain antibody.

47. The composition of claim 42, wherein the isolated antibody or portion thereof is a Fab fragment.

48. The composition of claim 42, wherein the antibody or portion thereof is labeled.

49. The composition of claim 48 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

50. An isolated cell that produces the antibody of claim 30.

51. A hybridoma that produces the antibody of claim 30.

52. A hybridoma that produces the antibody of claim 35.

53. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the isolated antibody or portion thereof of claim 30; and (b) detecting **NKEF** C protein in the biological sample.

54. The method of claim 53 wherein the isolated antibody or portion thereof is a monoclonal antibody.

55. The method of claim 53 wherein the isolated antibody or portion thereof is a polyclonal antibody.

56. The method of claim 53 wherein the isolated antibody or portion

thereof is a chimeric antibody.

57. The method of claim 53 wherein the isolated antibody or portion thereof is a single chain antibody.

58. The method of claim 53 wherein the antibody is a Fab fragment.

59. The method of claim 53 wherein the antibody is a labeled antibody.

60. The method of claim 59 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

61. An antibody or portion thereof produced by immunizing an animal with a protein selected from the group consisting of: (a) a protein whose sequence comprises amino acid residues +1 to +271 of SEQ ID NO:2; (b) a protein whose sequence comprises 30 contiguous amino acid residues of SEQ ID NO:2; and (c) a protein whose sequence comprises 50 contiguous amino acid residues of SEQ ID NO:2; wherein said antibody or portion thereof specifically binds to the amino acid sequence of SEQ ID NO:2.

62. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (a).

63. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (b).

64. The antibody or portion thereof of claim 61 produced by immunizing an animal with protein (c).

65. An isolated antibody or portion thereof that specifically binds to a protein whose sequence consists of the amino acid sequence of the mature form of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

66. The antibody or portion thereof of claim 65 wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

67. The antibody or portion thereof of claim 65 which is a monoclonal antibody.

68. The antibody or portion thereof of claim 65 which is a polyclonal antibody.

69. The antibody or portion thereof of claim 65 which is a chimeric antibody.

70. The antibody or portion thereof of claim 65 which is a single chain antibody.

71. The antibody or portion thereof of claim 65 which is a Fab fragment.

72. The antibody or portion thereof of claim 65 which is labeled.

73. The antibody of claim 72 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

74. A composition comprising the antibody or portion thereof of claim 65 and a carrier.

75. The composition of claim 74, wherein the antibody or portion thereof is a monoclonal antibody.

76. The composition of claim 74, wherein the antibody or portion thereof is a chimeric antibody.

77. The composition of claim 74, wherein the antibody or portion thereof is a single chain antibody.

78. The composition of claim 74, wherein the antibody or portion thereof is a Fab fragment.

79. The composition of claim 74, wherein the antibody or portion thereof is labeled.

80. The composition of claim 79 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

81. An isolated cell that produces the antibody of claim 65.

82. A hybridoma that produces the antibody of claim 65.

83. A hybridoma that produces the antibody of claim 67.

84. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 65; and (b) detecting the **NKEF** C protein in the biological sample.

85. The method of claim 84 wherein the antibody is a monoclonal antibody.

86. The method of claim 84 wherein the antibody is a polyclonal antibody.

87. The method of claim 84 wherein the antibody is a chimeric antibody.

88. The method of claim 84 wherein the antibody is a single chain antibody.

89. The method of claim 84 wherein the antibody is a Fab fragment.

90. The method of claim 84 wherein the antibody is a labeled antibody.

91. The method of claim 90 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

92. An isolated antibody or portion thereof produced by immunizing an animal with a protein whose sequence comprises the amino acid sequence of the mature form of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; wherein said antibody or portion thereof specifically binds to the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97103.

93. An isolated antibody or portion thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose sequence consists of the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; (b) a protein whose sequence consists of 30 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; and (c) a protein whose sequence consists of 50 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

94. The isolated antibody or portion thereof of claim 93 that specifically binds protein (a).

95. The isolated antibody or portion thereof of claim 93 that specifically binds protein (b).

96. The isolated antibody or portion thereof of claim 93 that specifically binds protein (c).

97. The isolated antibody or portion thereof of claim 93, wherein said protein specifically bound by said antibody or portion thereof is glycosylated.

98. The isolated antibody or portion thereof of claim 93, which is a monoclonal antibody.

99. The isolated antibody or portion thereof of claim 93, which is a polyclonal antibody.

100. The isolated antibody or portion thereof of claim 93, which is a chimeric antibody.

101. The isolated antibody or portion thereof of claim 93 which is a single chain antibody.

102. The isolated antibody or portion thereof of claim 93 which is a Fab fragment.

103. The isolated antibody or portion thereof of claim 93 which is labeled.

104. The isolated antibody or portion thereof of claim 103 wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

105. A composition comprising the isolated antibody or portion thereof of claim 93 and a carrier.

106. The composition of claim 105, wherein the antibody or portion thereof is a monoclonal antibody.

107. The composition of claim 105, wherein the antibody or portion thereof is a polyclonal antibody.

108. The composition of claim 105, wherein the antibody or portion thereof is a chimeric antibody.

109. The composition of claim 105, wherein the antibody or portion thereof is a single chain antibody.

110. The composition of claim 105, wherein the antibody or portion thereof is a Fab fragment.

111. The composition of claim 105, wherein the antibody or portion thereof is labeled.

112. The composition of claim 111, wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

113. An isolated cell that produces the isolated antibody or portion thereof of claim 93.

114. A hybridoma that produces the antibody of claim 93.

115. A hybridoma that produces the antibody of claim 98.

116. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample from a test subject with the isolated antibody or portion thereof of claim 93; and (b) detecting **NKEF** C protein in the biological sample.

117. The method of claim 116, wherein the antibody or portion thereof is a monoclonal antibody.

118. The method of claim 116, wherein the antibody or portion thereof is a polyclonal antibody.

119. The method of claim 116, wherein the antibody or portion thereof is a chimeric antibody.

120. The method of claim 116, wherein the antibody or portion thereof is a single chain antibody.

121. The method of claim 116, wherein the antibody or portion thereof is a Fab fragment.

122. The method of claim 116, wherein the antibody or portion thereof is labeled.

123. The method of claim 122, wherein the label is selected from the group consisting of: (a) an enzyme label; (b) a radioisotope; and (c) a fluorescent label.

124. An antibody or portion thereof produced by immunizing an animal with a protein selected from the group consisting of: (a) a protein whose sequence comprises the amino acid sequence of the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; (b) a protein whose sequence comprises at least 30 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; and (c) a protein whose sequence comprises at least 50 contiguous amino acid residues of a polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157; wherein said antibody or portion thereof specifically binds to the polypeptide encoded by the cDNA contained in ATCC® Deposit No. 97157.

125. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (a).

126. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (b).

127. (New) The antibody or portion thereof of claim 124 produced by immunizing an animal with protein (c).

128. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 1.

129. The method of claim 128, wherein the antibody is a monoclonal antibody.

130. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 30.

131. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 65.

132. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 93.

IN **Gentz, Reiner**, Silver Spring, MD, UNITED STATES|
AI US 2001-911346 A1 20010724 (9) <--
SUMM . . . the present invention has been putatively identified as a

to as "**NKEF** C." The invention also relates to inhibiting the action of such polypeptides.

SUMM [0006] Two **NKEF** genes (**NKEF**-A and B) from a K562 erythroleukemia cell cDNA library have recently been cloned (Shau, H., et al., Immunogenetics, 40:129-134 (1994)).. . . other (88% identical at the amino acid level, 71% identical in nucleotide sequence). It is not clear whether the dimeric **NKEF** is a homo- or hetero-dimer of the A or B peptides in vivo. **NKEF** A and **NKEF** B are differentially expressed in different tissues. **NKEF** A and **NKEF** B have similar antioxidant activity, but **NKEF** A has higher NK enhancing activity than **NKEFB**. Transfecting **NKEF** DNA into different cells resulted in cell-type-dependent enhanced cell proliferation or growth inhibition.

SUMM . . . proposed antioxidant genes are highly conserved from bacteria to mammals while mammals have been found to carry more than one **NKEF**-related gene, bacteria and yeast have been found to carry only one copy (Sauri, H., et al.). Members of this family have been described as thiol-specific antioxidants. These genes (**NKEF**-A and B) encode recombinant proteins which possess antioxidant function in the protection of protein and DNA from oxidative damage. **NKEF** is a 44 kD protein isolated from red blood cell cytosol that increases NK cell cytotoxicity to tumor target cells (Shau, H., et al., Cell. Immunol., 147, 1-11 (1993)). **NKEF** is a dimer protein composed of two approximately 22 kD monomers linked by disulphide bonds.

SUMM [0008] Two of the other **NKEF**-related proteins are human thiol-specific antioxidant protein (HPRP) isolated from a hippocampus cDNA library, and the proliferation-associated gene (PAG), found to be hyperexpressed in transformed cells. HPRP is 95% identical to **NKEF** B by nucleotide sequences, and 93% identical by amino acid sequence. Alignment with **NKEF**-related proteins in other species suggested that **NKEF** B and HPRP are the same. PAG shares 98% identity with **NKEF** A by nucleotide sequence, and 97% at the amino acid level, and may be identical to **NKEFA**.

SUMM [0009] In mice, the two homologous genes are MSP23 and MER5. MER5 is 61% identical to **NKEF** A in amino acid sequence and 64% identical to **NKEFB**. Even more striking is MSP23, which is 93% identical to **NKEF** A and 76% identical to **NKEFB**. MSP23 is induced by oxidative stress in mouse macrophage. MER5 is hyperexpressed in murine erythroleukemic cells, and is necessary for differentiation in those cells. **NKEF** and **NKEF**-related proteins show no sequence homology to other known antioxidants, such as catalase, superoxide dismutase, or glutathione peroxidase, nor do they. . .

SUMM [0011] The polypeptide of the present invention has been putatively identified as a **natural killer enhancing factor** C due to its amino acid sequence homology with human **natural killer enhancing factor**. This identification has been made as a result of amino acid sequence homology.

SUMM [0018] In accordance with another aspect of the present invention, there are provided **NKEF** C agonist compounds which mimic **NKEF** C and bind to **NKEF** C receptors to elicit the biological functions of wild-type **NKEF** C.

DRWD . . . sequence homology between the polypeptide of the present invention (top comparative line of each row, from SEQ ID NO:2), human **NKEF** A (second comparative line of each row, SEQ ID NO:14), **NKEF** B (third comparative line of each row, SEQ ID NO:15), MER5 (fourth comparative line of each row, SEQ ID NO:. . .

DRWD [0026] FIG. 3 illustrates the growth inhibitory activity of **NKEF** C against HL60 human promyelocytic leukemia cells.

DRWD [0027] FIG. 4 illustrates the growth inhibitory activity of **NKEF** C against Jurkat human T-cell leukemia cells.

DRWD [0028] FIG. 5 illustrates the effect of **NKEF** C on VSV lytic infection.

DETD . . . leader sequence such that the mature protein comprises 241 amino acids. The protein exhibits the highest degree of homology to **NKEF** B expressed from NK-sensitive erythroleukemic cell line K 562, as shown in Sauri, H., et al. with 68.182% identity and. . .

DETD [0041] Fragments of the full length **NKEF** C gene may be used as a hybridization probe for a cDNA library to isolate the full length gene

and to isolate other genes which have a high sequence similarity to the **NKEF** C gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for. . . identify a CDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **NKEF** C gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the **NKEF** C gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to. . .

DETD [0046] The present invention further relates to an **NKEF** C polypeptide which has the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or which has the amino. . .

DETD [0078] The **NKEF** C polypeptide of the present invention has been shown to significantly augment NK cell-mediated cytotoxicity when added at the initiation of cytotoxicity assays and **NKEF**, accordingly, may be employed to regulate NK function.

DETD [0079] The **NKEF** C polypeptide may be employed to enhance NK activity and therefore deter cancer development in the body. The **NKEF** C polypeptide may also be employed for immunoregulation of NK activity and may be important for cells in coping with. . .

DETD [0080] The **NKEF** C polypeptide of the present invention may also be employed to prevent inflammation.

DETD [0081] The **NKEF** C polypeptide of the present invention may also be employed to prevent NK- B activity and prevent viral transcription and. . . and therefore induce viral transcription. Accordingly, Human immunodeficiency virus type 1 (HIV-1) and HDLV-1 may also be treated with the **NKEF** C polypeptide of the present invention.

DETD [0084] This invention provides a method for identification of the receptor for the **NKEF** C polypeptide. The gene encoding the receptor can be identified by numerous methods known to those of skill in the. . . 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the **NKEF** C polypeptide, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the **NKEF** C polypeptide. Transfected cells which are grown on glass slides are exposed to labeled **NKEF** C polypeptide. The **NKEF** C polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a. . .

DETD . . . compounds to identify those which bind to and activate and those which bind to and inhibit the receptor for the **NKEF** C polypeptides. As an example, a mammalian cell or membrane preparation expressing the **NKEF** C receptor is incubated with a labeled compound to be tested. The compound may be labeled by a variety of. . . means known in the art, for example, by radioactivity. The ability of the compound to bind to and activate the **NKEF** C receptor could then be measured by the response of a known second messenger system. Such second messenger systems include,. . . are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis. For instance, an effective agonist binds to the **NKEF** C receptor and elicits a second messenger response while an effective antagonist binds to the receptor but does not elicit. . .

DETD . . . oligopeptide, which binds to the polypeptide. Alternatively, a potential antagonist may be a closely related protein which binds to the **NKEF** C receptor, however, they are inactive forms of the polypeptide and thereby prevent the action of **NKEF** C since receptor sites are occupied.

DETD . . . et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of **NKEF** C. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into **NKEF** C polypeptide (Antisense-Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)).. . . be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **NKEF** C.

DETD [0093] The **NKEF** C polypeptides and agonists and antagonists which are

polypeptides may also be employed in accordance with the present invention by. . .

DETD [0101] This invention is also related to the use of the **NKEF** C gene as a diagnostic. Detection of a mutated form of **NKEF** C will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of **NKEF** C for example, tumors and viral infections.

DETD [0102] Individuals carrying mutations in the human **NKEF** C gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be. . . cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding **NKEF** C can be used to identify and analyze **NKEF** C mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **NKEF** C RNA or alternatively, radiolabeled **NKEF** C antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences. . .

DETD [0108] The present invention also relates to a diagnostic assay for detecting altered levels of **NKEF** C protein in various tissues since over-expression compared to normal control tissue samples can detect the presence of a tumor or viral infection. Assays used to detect levels of **NKEF** C protein in a sample derived from a host are well-known to those of skill in the art and include. . . assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the **NKEF** C antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter. . . serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **NKEF** C proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked. . . peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **NKEF**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of **NKEF** C protein present in a given volume of patient sample when compared against a standard curve.

DETD [0109] A competition assay may be employed wherein antibodies specific to **NKEF** C are attached to a solid support and labeled **NKEF** C and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of **NKEF** C in the sample.

DETD [0129] Bacterial Expression and Purification of soluble **NKEF**

DETD [0130] The DNA sequence encoding **NKEF**, ATCC No. 97157, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the **NKEF** C protein and the vector sequences 3' to **NKEF** C. Additional nucleotides corresponding to **NKEF** C were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primers used for the full length sequence with His-tag has the sequence 5' GCGCGGATCCATGGAGGCGCTGCCCTGCT 3' (SEQ ID NO:3) contains a BamHI restriction enzyme site followed by **NKEF** C coding sequence starting from the presumed terminal amino acid of the processed protein and without the His-tag 5' CGCCCATGGAGGCGCTGCCCCTG 3' (SEQ ID NO:4) and contains a NcoI site. The 5' primer used for the **NKEF** C sequence without the leader sequence and without the His-tag is 5' CGCCCATGGCTGGAGCTGTGCAGGG 3' (SEQ ID NO:7) and has a. . . used were as follows: 5' CGCGTCTAGATCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:6) which contains complementary sequences to an XbaI site followed by **NKEF** C coding sequence; and 5' CGCGTCTAGATCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:[7] 6. The restriction enzyme sites correspond to the restriction enzyme. . . then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized **NKEF** C was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **NKEF** C was

eluted from the column in 6 molar guanidine HCl pH 6.5 and for the purpose of renaturation adjusted. . .

DETD [0131] Cloning and Expression of **NKEF** C Using the Baculovirus Expression System

DETD [0132] The DNA sequence encoding the full length **NKEF** C protein, ATCC No. 97157, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the. . .

DETD . . . signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987) and nucleotides of the **NKEF** C gene; and the 3' primer has the sequence 5' CGCGGATCCTCAATTCAGTTTATCGAAATAC 3' (SEQ ID NO:9) and contains the cleavage site for the restriction endonuclease BamHi and nucleotides complementary to the 3' non-translated sequence of the **NKEF** C gene.

DETD [0136] The vectors pA2-GP and pA2 (modifications of pVL941 vector, discussed below) are used for the expression of the **NKEF** C protein using the baculovirus expression system (for review see: Summers, M. D. and Smith, G. E. 1987, A manual. . .

DETD . . . with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacNKEF) with the **NKEF** C gene using the enzyme BamHI. The sequence of the cloned fragment was confirmed by DNA sequencing.

DETD . . . Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**NKEF** C at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900. . .

DETD [0144] Expression of Recombinant **NKEF** C in COS cells

DETD [0145] The expression of plasmid, **NKEF** C HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3). . . 4) CMV promoter followed by a polylinker region, an SV40 intron and polyadenylation site. A DNA fragment encoding the entire **NKEF** C precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of. . .

DETD [0147] The DNA sequence encoding **NKEF**, ATCC No. 97157, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GCGCGGATCCACCATGGAGGCGCTG 3' (SEQ ID NO:12) contains a BamHI site followed by 12 nucleotides of **NKEF** C coding sequence starting from the initiation codon; the 3' sequence 5' GCGCTCTAGATCAAGCGTAGTCTGGGACGTCGTAT GGGTAATTCAGTTTATC 3' (SEQ ID NO:13) contains complementary sequences to an XbaI site, translation stop codon, HA tag and the last 12 nucleotides of the **NKEF** C coding sequence (not including the stop codon). Therefore, the PCR product contains a BamHi site, **NKEF** C coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag,. . . isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant **NKEF**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **NKEF** C HA protein was detected by radio-labelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring. . .

DETD [0155] Growth Inhibitory Activity of **NKEF** C Against Human Leukemia Cells

DETD [0156] Two-fold serial dilution of purified **NKEF** C starting from 100 ng/ml were made in RPMI 1640 medium with 0.5% FBS. HL60 or Jurkat cells were harvested. . .

DETD [0157] **Antiviral** Activity of **NKEF** C against Vesicular Stomatitis Virus (VSV)

DETD [0158] The cytopathic effect reduction (CPER) assay is employed to measure the protective effect of **NKEF** C on the infection and cytopathic process of vesicular stomatitis virus (VSV) to normal human dermal fibroblasts (NHDF) from foreskin (Clonetics). In this experiment we performed serial dilution of **NKEF** C at a 1:2 ratio and extended the dilution starting from 3 μg/ml to 6 ng/ml final concentration. The positive. . . 100 μl. In addition, we maintained a negative (untreated) mock control. Semi-purified (70%) protein isolated from E. Coli expressing the **NKEF** C protein was employed in this study. The

NIH/ cells were seeded at 2x10 and incubated overnight to reach confluence.. . . scored for CPE by estimating the percentage of cells surviving on the -microtiter plate. The figure demonstrates a mean effective **NKEF** C concentration equal to 100 ng/ml.

21. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 1; and (b) detecting the **NKEF** C protein in the biological sample.

53. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the isolated antibody or portion thereof of claim 30; and (b) detecting **NKEF** C protein in the biological sample.

84. A method of detecting **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample with the antibody or portion thereof of claim 65; and (b) detecting the **NKEF** C protein in the biological sample.

116. A method of assaying **NKEF** C protein in a biological sample comprising: (a) contacting the biological sample from a test subject with the isolated antibody or portion thereof of claim 93; and (b) detecting **NKEF** C protein in the biological sample.

128. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 1.

130. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 30.

131. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 65.

132. A method of treating a patient having need of a reduced level of **NKEF** C protein, comprising administering to said patient the antibody or portion thereof of claim 93.

L34 ANSWER 26 OF 39 USPATFULL on STN
2001:162839 Natural killer cell enhancing factor C.
Ni, Jiar, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6294164 B1 20010925
APPLICATION: US 1999-407891 19990929 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.
CLM What is claimed is:
1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: (a) an amino acid sequence comprising residues +1 to +271 of SEQ ID NO:2; (b) an amino acid sequence

sequence comprising residues +31 to +271 of SEQ ID NO:2.

2. The isolated polypeptide of claim 1 which comprises amino acid sequence (a).

3. The isolated polypeptide of claim 1 which comprises amino acid sequence (b).

4. The isolated polypeptide of claim 1 which comprises amino acid sequence (c).

5. The isolated polypeptide of claim 1 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

6. A composition comprising the isolated polypeptide of claim 1 and a pharmaceutically acceptable carrier.

7. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: (a) an amino acid sequence of the full length polypeptide encoded by the cDNA in ATCC Deposit No. 97157; (b) an amino acid sequence of the full length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 97157; and (c) an amino acid sequence of the mature polypeptide encoded by the cDNA in ATCC Deposit No. 97157.

8. The isolated polypeptide of claim 7 which comprises amino acid sequence (a).

9. The isolated polypeptide of claim 7 which comprises amino acid sequence (b).

10. The isolated polypeptide of claim 7 which comprises amino acid sequence (c).

11. The isolated polypeptide of claim 7 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

12. A composition comprising the isolated polypeptide of claim 7 and a pharmaceutically acceptable carrier.

13. An isolated polypeptide comprising at least 30 contiguous amino acid residues of SEQ ID NO:2.

14. The isolated polypeptide of claim 13 further comprising at least 50 contiguous amino acid residues of SEQ ID NO:2.

15. The isolated polypeptide of claim 13 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

16. A composition comprising the isolated polypeptide of claim 13 and a pharmaceutically acceptable carrier.

17. The isolated polypeptide of claim 13 wherein said polypeptide has growth inhibitory activity.

18. The isolated polypeptide of claim 13 wherein said polypeptide has anti-viral activity.

19. An isolated polypeptide comprising at least 30 contiguous amino acid residues encoded by the cDNA in ATCC Deposit No. 97157.

20. The isolated polypeptide of claim 19 further comprising at least 50 contiguous amino acid residues encoded by the cDNA in ATCC Deposit No. 97157.

21. The isolated polypeptide of claim 19 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

22. A composition comprising the isolated polypeptide of claim 19 and a pharmaceutically acceptable carrier.

23. The isolated polypeptide of claim 19 wherein said polypeptide has growth inhibitory activity.

24. The isolated polypeptide of claim 19 wherein said polypeptide has anti-viral activity.

25. An isolated polypepride comprising a first amino acid sequence 95% or more identical to a second amino acid sequence selected from the group consisting of: (a) amino acids +1 to +271 of SEQ ID NO:2; (b) amino acids +2 to +271 of SEQ ID NO:2; and (c) amino acids +31 to +271 of SEQ ID NO:2.

26. The isolated polypeptide of claim 25 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (a).

27. The isolated polypeptide of claim 25 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (b).

28. The isolated polypeptide of claim 25 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (c).

29. The isolated polypeptide of claim 25 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

30. A composition comprising the isolated polypeptide of claim 25 and a pharmaceutically acceptable carrier.

31. An isolated polypeptide comprising a first amino acid sequence 95% or more identical to a second amino acid sequence selected from the group consisting of: (a) an amino acids sequence of the full length polypeptide encoded by the cDNA in ATCC Deposit No 97157; (b) an amino acids sequence of the full length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 97157; and (c) an amino acid sequence of the manture polypeptide encoded by the cDNA in ATCC Deposit No. 97157.

32. The isolated polypeptide of claim 31 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (a).

33. The isolated polypeptide of claim 31 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (b).

34. The isolated polypeptide of claim 31 wherein said first amino acid sequence is 95% identical to said second amino acid sequence (c).

35. The isolated polypeptide of claim 31 wherein said amino acid sequence further comprises a heterologous polypeptide sequence.

36. A composition comprising the isolated polypeptide of claim 31 and a pharmaceutically acceptable carrier.

37. An isolated polypeptide comprising a fragment of SEQ ID NO:2, wherein said fragment has growth inhibitory activity.

38. An isolated polypeptide comprising a fragment of SEQ ID NO:2, wherein said fragment has anti-viral activity.

IN **Gentz, Reiner**, Silver Spring, MD, United States|
AI US 1999-407891 19990929 (9) <--
SUMM . . . the present invention has been putatively identified as a

natural killer cell enhancing factor C, sometimes hereinafter referred to as "**NKEF** C." The invention also relates to inhibiting the action of such polypeptides.

SUMM Two **NKEF** genes (**NKEF**-A and B) from a K562 erythroleukemia cell cDNA library have recently been cloned (Shau, H., et al., Immunogenetics, 40:129-134 (1994)).. . . other (88% identical at the amino acid level, 71% identical in nucleotide sequence). It is not clear whether the dimeric **NKEF** is a homo- or hetero-dimer of the A or B peptides in vivo. **NKEF** A and **NKEF** B are differentially expressed in different tissues. **NKEF** A and **NKEF** B have similar antioxidant activity, but **NKEF** A has higher NK enhancing activity than **NKEFB**. Transfecting **NKEF** DNA into different cells resulted in cell-type-dependent enhanced cell proliferation or growth inhibition.

SUMM . . . proposed antioxidant genes are highly conserved from bacteria to mammals while mammals have been found to carry more than one **NKEF**-related gene, bacteria and yeast have been found to carry only one copy (Sauri, H., et al.). Members of this family have been described as thiol-specific antioxidants. These genes (**NKEF**-A and B) encode recombinant proteins which possess antioxidant function in the protection of protein and DNA from oxidative damage. **NKEF** is a 44 kD protein isolated from red blood cell cytosol that increases NK cell cytotoxicity to tumor target cells (Shau, H., et al., Cell. Immunol., 147, 1-11 (1993)). **NKEF** is a diner protein composed of two approximately 22 kD monomers linked by disulphide bonds.

SUMM Two of the other **NKEF**-related proteins are human thiol-specific antioxidant protein (HPRP) isolated from a hippocampus cDNA library, and the proliferation-associated gene (PAG), found to be hyperexpressed in transformed cells. HPRP is 95% identical to **NKEF** B by nucleotide sequences, and 93% identical by amino acid sequence. Alignment with **NKEF**-related proteins in other species suggested that **NKEF** B and HPRP are the same. PAG shares 98% identity with **NKEF** A by nucleotide sequence, and 97% at the amino acid level, and may be identical to **NKEFA**.

SUMM In mice, the two homologous genes are MSP23 and MER5. MER5 is 61% identical to **NKEF** A in amino acid sequence and 64% identical to **NKEFB**. Even more striking is MSP23, which is 93% identical to **NKEF** A and 76% identical to **NKEFB**. MSP23 is induced by oxidative stress in mouse macrophage. MER5 is hyperexpressed in murine erythroleukemic cells, and is necessary for differentiation in those cells. **NKEF** and **NKEF**-related proteins show no sequence homology to other known antioxidants, such as catalase, superoxide dismutase, or glutathione peroxidase, nor do they. . .

SUMM The polypeptide of the present invention has been putatively identified as a **natural killer enhancing factor** C due to its amino acid sequence homology with human **natural killer enhancing factor**. This identification has been made as a result of amino acid sequence homology.

SUMM In accordance with another aspect of the present invention, there are provided **NKEF** C agonist compounds which mimic **NKEF** C and bind to **NKEF** C receptors to elicit the biological functions of wild-type **NKEF** C.

DRWD . . . sequence homology between the polypeptide of the present invention (top comparative line of each row, from SEQ ID NO:2), human **NKEF** A (second comparative line of each row, SEQ ID NO: 14), **NKEF** B (third comparative line of each row, SEQ ID NO:15), MER5 (fourth comparative line of each row, SEQ ID NO:16). . .

DRWD FIG. 3 illustrates the growth inhibitory activity of **NKEF** C against HL60 human promyelocytic leukemia cells.

DRWD FIG. 4 illustrates the growth inhibitory activity of **NKEF** C against Jurkat human T-cell leukemia cells.

DRWD FIG. 5 illustrates the effect of **NKEF** C on VSV lytic infection.

DETD . . . leader sequence such that the mature protein comprises 241 amino acids. The protein exhibits the highest degree of homology to **NKEF** B expressed from NK-sensitive erythroleukemic cell line K 562, as shown in Sauri, H., et al. with 68.182% identity and. . .

DETD Fragments of the full length **NKEF** C gene may be used as a hybridization probe for a cDNA library to isolate the full length gene

and to isolate other genes which have a high sequence similarity to the **NKEF** C gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for. . . identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **NKEF** C gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the **NKEF** C gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to. . .

DETD The present invention further relates to an **NKEF** C polypeptide which has the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or which has the amino. . .

DETD The **NKEF** C polypeptide of the present invention has been shown to significantly augment NK cell-mediated cytotoxicity when added at the initiation of cytotoxicity assays and **NKEF**, accordingly, may be employed to regulate NK function.

DETD The **NKEF** C polypeptide may be employed to enhance NK activity and therefore deter cancer development in the body. The **NKEF** C polypeptide may also be employed for immunoregulation of NK activity and may be important for cells in coping with. . .

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent inflammation.

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent NK-κB activity and prevent viral transcription and therefore. . . and therefore induce viral transcription. Accordingly, Human immunodeficiency virus type 1 (HIV-1) and HDLV-1 may also be treated with the **NKEF** C polypeptide of the present invention.

DETD This invention provides a method for identification of the receptor for the **NKEF** C polypeptide. The gene encoding the receptor can be identified by numerous methods known to those of skill in the. . . 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the **NKEF** C polypeptide, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the **NKEF** C polypeptide. Transfected cells which are grown on glass slides are exposed to labeled **NKEF** C polypeptide. The **NKEF** C polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a. . .

DETD . . . compounds to identify those which bind to and activate and those which bind to and inhibit the receptor for the **NKEF** C polypeptides. As an example, a mammalian cell or membrane preparation expressing the **NKEF** C receptor is incubated with a labeled compound to be tested. The compound may be labeled by a variety of. . . means known in the art, for example, by radioactivity. The ability of the compound to bind to and activate the **NKEF** C receptor could then be measured by the response of a known second messenger system. Such second messenger systems include,. . . are not limited to, CAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis. For instance, an effective agonist binds to the **NKEF** C receptor and elicits a second messenger response while an effective antagonist binds to the receptor but does not elicit. . .

DETD . . . oligopeptide, which binds to the polypeptide. Alternatively, a potential antagonist may be a closely related protein which binds to the **NKEF** C receptor, however, they are inactive forms of the polypeptide and thereby prevent the action of **NKEF** C since receptor sites are occupied.

DETD . . . Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251:1360 (1991)), thereby preventing transcription and the production of **NKEF** C. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into **NKEF** C polypeptide (Antisense--Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)).. . . be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **NKEF** C.

DETD The **NKEF** C polypeptides and agonists and antagonists which are

polypeptides may also be employed in accordance with the present invention by. . .

DETD This invention is also related to the use of the **NKEF** C gene as a diagnostic. Detection of a mutated form of **NKEF** C will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of **NKEF** C for example, tumors and viral infections.

DETD Individuals carrying mutations in the human **NKEF** C gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be. . . cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding **NKEF** C can be used to identify and analyze **NKEF** C mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **NKEF** C RNA or alternatively, radiolabeled **NKEF** C antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences. . .

DETD The present invention also relates to a diagnostic assay for detecting altered levels of **NKEF** C protein in various tissues since over-expression compared to normal control tissue samples can detect the presence of a tumor or viral infection. Assays used to detect levels of **NKEF** C protein in a sample derived from a host are well-known to those of skill in the art and include. . . assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the **NKEF** C antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter. . . serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **NKEF** C proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked. . . peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **NKEF**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of **NKEF** C protein present in a given volume of patient sample when compared against a standard curve.

DETD A competition assay may be employed wherein antibodies specific to **NKEF** C are attached to a solid support and labeled **NKEF** C and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of **NKEF** C in the sample.

DETD Bacterial Expression and Purification of Soluble **NKEF**

DETD The DNA sequence encoding **NKEF**, ATCC No. 97157, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the **NKEF** C protein and the vector sequences 3' to **NKEF** C. Additional nucleotides corresponding to **NKEF** C were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primers used for the full length sequence. . . with His-tag has the sequence 5' GCGCGGATCC ATGGAGGCGCTGCCCTGCT 3' (SEQ ID NO:3) contains a BamHI restriction enzyme site followed by **NKEF** C coding sequence starting from the presumed terminal amino acid of the processed protein and without the His-tag 5' CGCCCATGGAGGCGCTGCCCCTG 3' (SEQ ID NO:4) and contains a NcoI site. The 5' primer used for the **NKEF** C sequence without the leader sequence and without the His-tag is 5' CGCCCATGGCTGG AGCTGTGCAGGG 3' (SEQ ID NO:5) and has. . . used were as follows: 5' CGCGTCTAGATCAATTCAGTTTATCGAAATA CTTCAGC 3' (SEQ ID NO:6) which contains complementary sequences to an XbaI site followed by **NKEF** C coding sequence; and 5' CGCGTCTAGATCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:6). The restriction enzyme sites correspond to the restriction enzyme sites. . . then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized **NKEF** C was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **NKEF** C was eluted from the column in 6 molar

guanidine HCl pH 8.0 and for the purpose of renaturation adjusted. . .

DETD Cloning and Expression of **NKEF** C Using the Baculovirus Expression System

DETD The DNA sequence encoding the full length **NKEF** C protein, ATCC No. 97157, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the. . .

DETD . . . signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987) and nucleotides of the **NKEF** C gene; and the 3' primer has the sequence 5' CGCGUTCCTCAATTCAGTTTATCGAAATAC 3' (SEQ ID NO:9) and contains the cleavage site for the restriction endonuclease BamHI and nucleotides complementary to the 3' non-translated sequence of the **NKEF** C gene.

DETD The vectors pA2-GP and pA2 (modifications of pVL941 vector, discussed below) are used for the expression of the **NKEF** C protein using the baculovirus expression system (for review see: Summers, M. D. and Smith, G. E. 1987, A manual. . .

DETD . . . with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacNKEF) with the **NKEF** C gene using the enzyme BamHI. The sequence of the cloned fragment was confirmed by DNA sequencing.

DETD Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**NKEF** C at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900. . .

DETD Expression of Recombinant **NKEF** C in COS Cells

DETD The expression of plasmid, **NKEF** C HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3). . . 4) CMV promoter followed by a polylinker region, an SV40 intron and polyadenylation site. A DNA fragment encoding the entire **NKEF** C precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of. . .

DETD The DNA sequence encoding **NKEF**, ATCC No. 97157, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GCGCGGATCCACCATGGAGGCGCTG 3' (SEQ ID NO:12) contains a BamHI site followed by 12 nucleotides of **NKEF** C coding sequence starting from the initiation codon; the 3' sequence 5' GCGCTCTAGATCAAGCGTAGTCTGGGACGTCGTAT GGGTAATTCAGTTTATC 3' (SEQ ID NO:13) contains complementary sequences to an XbaI site, translation stop codon, HA tag and the last 12 nucleotides of the **NKEF** C coding sequence (not including the stop codon). Therefore, the PCR product contains a BamHI site, **NKEF** C coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag,. . . isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant **NKEF**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, B. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **NKEF** C HA protein was detected by radiolabelling and immmoprecipitation method (B. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring. . .

DETD Growth Inhibitory Activity of **NKEF** C Against Human Leukemia Cells

DETD Two-fold serial dilution of purified **NKEF** C starting from 100 ng/ml were made in RPMI 1640 medium with 0.5% FBS. HL60 or Jurkat cells were harvested. . .

DETD **Antiviral** Activity of **NKEF** C Against Vesicular Stomatitis Virus (VSV)

DETD The cytopathic effect reduction (CPER) assay is employed to measure the protective effect of **NKEF** C on the infection and cytopathic process of vesicular stomatitis virus (VSV) to normal human dermal fibroblasts (NHDF) from foreskin (Clonetics). In this experiment we performed serial dilution of **NKEF** C at a 1:2 ratio and extended the dilution starting from 3 μg/ml to 6 ng/ml final concentration. The positive. . . 100 μl. In addition, we maintained a negative (untreated) mock control. Semi-purified (.about.70%) protein isolated from E. Coli expressing the **NKEF** C protein was employed in this study. The NHDF cells were seeded at 2×10^4 /well and incubated overnight to reach. . .
scored for CPE by estimating the percentage of cells surviving on the microtiter plate. The figure demonstrates a mean effective **NKEF** C

concentration equal to ~~about 100 ng/ml~~.

L34 ANSWER 27 OF 39 USPATFULL on STN
2001:102589 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, Gaithersburg, MD, United States
Yu, Guo-Liang, Darnestown, MD, United States
Gentz, Reiner, Silver Spring, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6255079 B1 20010703
APPLICATION: US 1995-467265 19950606 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.

CLM What is claimed is:

1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

2. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (a).

3. The polynucleotide of claim 2, which comprises nucleotides 31 to 843 of SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (b).

5. The polynucleotide of claim 4, which comprises nucleotides 34 to 843 of SEQ ID NO:1.

6. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (c).

7. The polynucleotide of claim 6, which comprises nucleotides 121-843.

8. The isolated nucleic acid molecule of claim 1 fused to a heterologous polynucleotide.

9. The isolated nucleic acid molecule of claim 8, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

10. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is DNA.

11. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide is double stranded.

12. A recombinant vector comprising the nucleic acid molecule of claim 1.

13. A recombinant host comprising the nucleic acid molecule of claim 1 operatively associated with a heterologous regulatory sequence.

14. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 13 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

15. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (d).

16. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (e).

17. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (f).

18. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (g).

19. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (h).

20. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (i).

21. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (j).

22. An isolated nucleic acid molecule consisting of a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO:1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

23. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (a).

24. The polynucleotide of claim 23, which comprises nucleotides 31 to 843 of SEQ ID NO:1.

25. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (b).

26. The polynucleotide of claim 25, which comprises nucleotides 81 to 843 of SEQ ID NO:1.

27. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (c).

28. The polynucleotide of claim 27, which comprises nucleotides 121-843.

29. The isolated nucleic acid molecule of claim 22 fused to a heterologous polynucleotide.

30. The isolated nucleic acid molecule of claim 29, wherein the heterologous polynucleotide encodes for a heterologous polypeptide.

31. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is DNA.

32. The isolated nucleic acid molecule of claim 22, wherein the polynucleotide is double stranded.

33. A recombinant vector comprising the nucleic acid molecule of claim 22.

34. A recombinant host comprising the nucleic acid molecule of claim 22 operatively associated with a heterologous regulatory sequence.

35. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 34 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

36. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (d).

37. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (e).

38. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (f).

39. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (g).

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41. The isolated nucleic acid molecule of claim 22, wherein said polynucleotide is (i).

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IN **Gentz, Reiner**, Silver Spring, MD, United States|
AI US 1995-467265 19950606 (8) <--
SUMM . . . the present invention has been putatively identified as a natural killer cell enhancing factor C, sometimes hereinafter referred to as "**NKEF** C." The invention also relates to inhibiting the action of such polypeptides.
SUMM Two **NKEF** genes (**NKEF**-A and B) from a K562 erythroleukemia cell cDNA library have recently been cloned (Shau, H., et al., Immunogenetics, 40:129-134 (1994)).. . . other (88% identical at the amino acid level, 71% identical in nucleotide sequence). It is not clear whether the dimeric **NKEF** is a homo- or hetero-dimer of the A or B peptides in vivo. **NKEF** A and **NKEF** B are differentially expressed in different tissues. **NKEF** A and **NKEF** B have similar antioxidant activity, but **NKEF** A has higher NK enhancing activity than **NKEFB**. Transfecting

NKEF B and A lines differed cells resulted in cell type dependent enhanced cell proliferation or growth inhibition.

SUMM . . . proposed antioxidant genes are highly conserved from bacteria to mammals while mammals have been found to carry more than one **NKEF**-related gene, bacteria and yeast have been found to carry only one copy (Sauri, H., et al.). Members of this family have been described as thiol-specific antioxidants. These genes (**NKEF**-A and B) encode recombinant proteins which possess antioxidant function in the protection of protein and DNA from oxidative damage. **NKEF** is a 44 kD protein isolated from red blood cell cytosol that increases NK cell cytotoxicity to tumor target cells (Shau, H., et al., Cell. Immunol., 147, 1-11 (1993)). **NKEF** is a dimer protein composed of two approximately 22 kD monomers linked by disulphide bonds.

SUMM Two of the other **NKEF**-related proteins are human thiol-specific antioxidant protein (HPRP) isolated from a hippocampus cDNA library, and the proliferation-associated gene (PAG), found to be hyperexpressed in transformed cells. HPRP is 95% identical to **NKEF** B by nucleotide sequences, and 93% identical by amino acid sequence. Alignment with **NKEF**-related proteins in other species suggested that **NKEF** B and HPRP are the same. PAG shares 98% identity with **NKEF** A by nucleotide sequence, and 97% at the amino acid level, and may be identical to **NKEFA**.

SUMM In mice, the two homologous genes are MSP23 and MER5. MER5 is 61% identical to **NKEF** A in amino acid sequence and 64% identical to **NKEFB**. Even more striking is MSP23, which is 93% identical to **NKEF** A and 76% identical to **NKEFB**. MSP23 is induced by oxidative stress in mouse macrophage. MER5 is hyperexpressed in murine erythroleukemic cells, and is necessary for differentiation in those cells. **NKEF** and **NKEF**-related proteins show no sequence homology to other known antioxidants, such as catalase, superoxide dismutase, or glutathione peroxidase, nor do they. . .

SUMM The polypeptide of the present invention has been putatively identified as a **natural killer enhancing factor** C due to its amino acid sequence homology with human **natural killer enhancing factor**. This identification has been made as a result of amino acid sequence homology.

SUMM In accordance with another aspect of the present invention, there are provided **NKEF** C agonist compounds which mimic **NKEF** C and bind to **NKEF** C receptors to elicit the biological functions of wild-type **NKEF** C.

DRWD . . . sequence homology between the polypeptide of the present invention (top comparative line of each row, from SEQ ID NO:2), human **NKEF** A (second comparative line of each row, SEQ ID NO:14), **NKEF** B (third comparative line of each row, SEQ ID NO:15), MER5 (fourth comparative line of each row, SEQ ID NO:16). . .

DRWD FIG. 3 illustrates the growth inhibitory activity of **NKEF** C against HL60 human promyelocytic leukemia cells.

DRWD FIG. 4 illustrates the growth inhibitory activity of **NKEF** C against Jurkat human T-cell leukemia cells.

DRWD FIG. 5 illustrates the effect of **NKEF** C on VSV lytic infection.

DETD . . . leader sequence such that the mature protein comprises 241 amino acids. The protein exhibits the highest degree of homology to **NKEF** B expressed from NK-sensitive erythroleukemic cell line K 562, as shown in Sauri, H., et al. with 68.182% identity and. . .

DETD Fragments of the full length **NKEF** C gene may be used as a hybridization probe for a cDNA library to isolate the full length gene and to isolate other genes which have a high sequence similarity to the **NKEF** C gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for. . . identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **NKEF** C gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the **NKEF** C gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to. . .

DETD The present invention further relates to an **NKEF** C polypeptide which has the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or which has

DETD The **NKEF** C polypeptide of the present invention has been shown to significantly augment NK cell-mediated cytotoxicity when added at the initiation of cytotoxicity assays and **NKEF**, accordingly, may be employed to regulate NK function.

DETD The **NKEF** C polypeptide may be employed to enhance NK activity and therefore deter cancer development in the body. The **NKEF** C polypeptide may also be employed for immunoregulation of NK activity and may be important for cells in coping with. . .

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent inflammation.

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent NK-κB activity and prevent viral transcription and therefore. . . and therefore induce viral transcription. Accordingly, Human immunodeficiency virus type 1 (HIV-1) and HDLV-1 may also be treated with the **NKEF** C polypeptide of the present invention.

DETD This invention provides a method for identification of the receptor for the **NKEF** C polypeptide. The gene encoding the receptor can be identified by numerous methods known to those of skill in the. . . 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the **NKEF** C polypeptide, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the **NKEF** C polypeptide. Transfected cells which are grown on glass slides are exposed to labeled **NKEF** C polypeptide. The **NKEF** C polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a. . .

DETD . . . compounds to identify those which bind to and activate and those which bind to and inhibit the receptor for the **NKEF** C polypeptides. As an example, a mammalian cell or membrane preparation expressing the **NKEF** C receptor is incubated with a labeled compound to be tested. The compound may be labeled by a variety of. . . means known in the art, for example, by radioactivity. The ability of the compound to bind to and activate the **NKEF** C receptor could then be measured by the response of a known second messenger system. Such second messenger systems include,. . . are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis. For instance, an effective agonist binds to the **NKEF** C receptor and elicits a second messenger response while an effective antagonist binds to the receptor but does not elicit. . .

DETD . . . oligopeptide, which binds to the polypeptide. Alternatively, a potential antagonist may be a closely related protein which binds to the **NKEF** C receptor, however, they are inactive forms of the polypeptide and thereby prevent the action of **NKEF** C since receptor sites are occupied.

DETD . . . Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251:1360 (1991)), thereby preventing transcription and the production of **NKEF** C. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into **NKEF** C polypeptide (Antisense--Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)).. . . be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **NKEF** C.

DETD The **NKEF** C polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by. . .

DETD This invention is also related to the use of the **NKEF** C gene as a diagnostic. Detection of a mutated form of **NKEF** C will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of **NKEF** C for example, tumors and viral infections.

DETD Individuals carrying mutations in the human **NKEF** C gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be. . . cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding **NKEF** C can be used to identify and analyze **NKEF** C mutations. For

example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **NKEF** C RNA or alternatively, radiolabeled **NKEF** C antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences. . .

DETD The present invention also relates to a diagnostic assay for detecting altered levels of **NKEF** C protein in various tissues since over-expression compared to normal control tissue samples can detect the presence of a tumor or viral infection. Assays used to detect levels of **NKEF** C protein in a sample derived from a host are well-known to those of skill in the art and include. . . assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the **NKEF** C antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter. . . serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **NKEF** C proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked. . . peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **NKEF**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of **NKEF** C protein present in a given volume of patient sample when compared against a standard curve.

DETD A competition assay may be employed wherein antibodies specific to **NKEF** C are attached to a solid support and labeled **NKEF** C and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of **NKEF** C in the sample.

DETD Bacterial Expression and Purification of Soluble **NKEF**

DETD The DNA sequence encoding **NKEF**, ATCC #97157, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the **NKEF** C protein and the vector sequences 3' to **NKEF** C. Additional nucleotides corresponding to **NKEF** C were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primers used for the full length sequence. . . with His-tag has the sequence 5' GCGCGGATCC ATGGAGGCGCTGCCCTGCT 3' (SEQ ID NO:3) contains a BamHI restriction enzyme site followed by **NKEF** C coding sequence starting from the presumed terminal amino acid of the processed protein and without the His-tag 5' CGCCCATGGAGGCGCTGCCCCTG 3' (SEQ ID NO:4) and contains a NcoI site. The 5' primer used for the **NKEF** C sequence without the leader sequence and without the His-tag is 5' CGCCCATGGCTGG AGCTGTGCAGGG 3' (SEQ ID NO:5) and has. . . used were as follows: 5' CGCGTCTAGATCAATTCAGTTTATCGAAATA CTTCAGC 3' (SEQ ID NO:6) which contains complementary sequences to an XbaI site followed by **NKEF** C coding sequence; and 5' CGCGTCTAGA TCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:6). The restriction enzyme sites correspond to the restriction enzyme. . . then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized **NKEF** C was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **NKEF** C was eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted. . .

DETD Cloning and Expression of **NKEF** C Using the Baculovirus Expression System

DETD The DNA sequence encoding the full length **NKEF** C protein, ATCC #97157, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

DETD . . . signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987) and nucleotides of the **NKEF** C gene; and the 3' primer has the sequence 5' CGCGGATCCTCAATTCAGTTTATCGAAATAC 3' (SEQ ID NO:9) and contains the cleavage site for the restriction endonuclease BamHI and nucleotides complementary to the 3' non-translated sequence of the **NKEF** C gene.

DETD [illegible] below) are used for the expression of the **NKEF** C protein using the baculovirus expression system (for review see: Summers, M. D. and Smith, G. E. 1987, A manual. . .

DETD . . . with T4 DNA ligase. E.coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacNKEF) with the **NKEF** C gene using the enzyme BamHI. The sequence of the cloned fragment was confirmed by DNA sequencing.

DETD Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**NKEF** C at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900. . .

DETD Expression of Recombinant **NKEF** C in COS Cells

DETD The expression of plasmid, **NKEF** C HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3). . . 4) CMV promoter followed by a polylinker region, an SV40 intron and polyadenylation site. A DNA fragment encoding the entire **NKEF** C precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of. . .

DETD The DNA sequence encoding **NKEF**, ATCC #97157, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GCGCGGATCCACCATGGAGGCGCTG 3' (SEQ ID NO:12) contains a BamHI site followed by 12 nucleotides of **NKEF** C coding sequence starting from the initiation codon; the 3' sequence 5' GCGCTCTAGATCAAGCGTAGTCTGGGACGTCGT ATGGGTAATTCAGTTTATC 3' (SEQ ID NO:13) contains complementary sequences to an XbaI site, translation stop codon, HA tag and the last 12 nucleotides of the **NKEF** C coding sequence (not including the stop codon). Therefore, the PCR product contains a BamHI site, **NKEF** C coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag,. . . isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant **NKEF**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **NKEF** C HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring. . .

DETD Growth Inhibitory Activity of **NKEF** C Against Human Leukemia Cells

DETD Two-fold serial dilution of purified **NKEF** C starting from 100 ng/ml were made in RPMI 1640 medium with 0.5% FBS. HL60 or Jurkat cells were harvested. . .

DETD **Antiviral** Activity of **NKEF** C against Vesicular Stomatitis Virus (VSV)

DETD The cytopathic effect reduction (CPER) assay is employed to measure the protective effect of **NKEF** C on the infection and cytopathic process of vesicular stomatitis virus (VSV) to normal human dermal fibroblasts (NHDF) from foreskin (Clonetics). In this experiment we performed serial dilution of **NKEF** C at a 1:2 ratio and extended the dilution starting from 3 μg/ml to 6 ng/ml final concentration. The positive. . . 100 μl. In addition, we maintained a negative (untreated) mock control. Semi-purified (.about.70%) protein isolated from E. coli expressing the **NKEF** C protein was employed in this study. The NHDF cells were seeded at 2×10^4 /well and incubated overnight to reach. . .
scored for CPE by estimating the percentage of cells surviving on the microtiter plate. The figure demonstrates a mean effective **NKEF** C concentration equal to .about.100 ng/ml.

. . . amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide. . .

. . . amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having

the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide. . .

L34 ANSWER 33 OF 39 USPATFULL on STN
1999:146312 Polynucleotides encoding natural killer cell enhancing factor C.
Ni, Jian, 305 W. Side Dr., Apt. #204, Gaithersburg, MD, United States 20878
Yu, Guo-Liang, 13524 Straw Bale La., Darnestown, MD, United States 20878
Gentz, Reiner, 13404 Fairland Park Dr., Silver Spring, MD, United States 20904
Rosen, Craig A., 22400 Rolling Hill Rd., Laytonsville, MD, United States 20882
US 5985612 19991116
APPLICATION: US 1995-467265 19950606 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A human natural killer cell enhancing factor C and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for preventing and/or treating viral infections, inflammation, neoplasia and damage from superoxide radicals. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases, for example, cancer, are also disclosed.
CLM What is claimed is:
1. An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of: (a) a polynucleotide encoding amino acids 1-271 of SEQ ID NO:2; (b) a polynucleotide encoding amino acids 2-271 of SEQ ID NO:2; (c) a polynucleotide encoding amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide encoding at least 30 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (g) a polynucleotide encoding at least 50 contiguous amino acids of SEQ ID NO:2 or the cDNA clone contained in ATCC Deposit No. 97157; (h) a polynucleotide of at least 30 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; (i) a polynucleotide of at least 50 contiguous nucleotides of SEQ ID NO: 1 or the cDNA clone contained in ATCC Deposit No. 97157; and (j) the complement of (a), (b), (c), (d), (e), (f), (g), (h), or (i).

2. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (a).

3. The polynucleotide of claim 2, which comprises nucleotides 31 to 843 of SEQ ID NO: 1.

4. The isolated nucleic acid molecule of claim 1, wherein said polynucleotide is (b).

5. The polynucleotide of claim 4, which comprises nucleotides 34 to 843 of SEQ ID NO: 1.

6. The isolated nucleic acid molecule of claim 3, wherein said polynucleotide is (c).

7. The polynucleotide of claim 6, which comprises nucleotides 121-843.

8. The isolated nucleic acid molecule of claim 1 fused to a heterologous polynucleotide.

9. The isolated nucleic acid molecule of claim 8, wherein the

heterologous polynucleotide encoded for a heterologous polypeptide.

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12. A recombinant vector comprising the nucleic acid molecule of claim 1.

13. A recombinant host comprising the nucleic acid molecule of claim 1 operatively associated with a heterologous regulatory sequence.

14. A method of producing a polypeptide comprising: (a) culturing the recombinant host cell of claim 13 under conditions such that a polypeptide is expressed from the nucleic acid molecule; and (b) recovering said polypeptide.

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IN **Gentz, Reiner**, 13404 Fairland Park Dr., Silver Spring, MD, United States 20904|

AI US 1995-467265 19950606 (8) <--

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SUMM In mice, the two homologous genes are MSP23 and MER5. MER5 is 61% identical to **NKEF** A in amino acid sequence and 64% identical to **NKEFB**. Even more striking is MSP23, which is 93% identical to **NKEF** A and 76% identical to **NKEFB**. MSP23 is induced by oxidative stress in mouse macrophage. MER5 is hyperexpressed in murine erythroleukemic cells, and is necessary for differentiation in those cells. **NKEF** and **NKEF**-related proteins show no sequence homology to other known antioxidants, such as catalase, superoxide dismutase, or glutathione peroxidase, nor do they. . .

SUMM The polypeptide of the present invention has been putatively identified as a **natural killer enhancing factor** C due to its amino acid sequence homology with human **natural killer enhancing factor**. This identification has been made as a result of amino acid sequence homology.

SUMM In accordance with another aspect of the present invention, there are provided **NKEF** C agonist compounds which mimic **NKEF** C and bind to **NKEF** C receptors to elicit the biological functions of wild-type **NKEF** C.

DRWD . . . homology between the polypeptide of the present invention (top comparative line of each row, from SEQ ID NO: 2), human **NKEF** A (second comparative line of each row, SEQ ID NO:14), **NKEF** B (third comparative line of each row, SEQ ID NO:15), MER5 (fourth comparative line of each row, SEQ ID NO:16). . .

DRWD FIG. 3 illustrates the growth inhibitory activity of **NKEF** C against HL60 human promyelocytic leukemia cells.

DRWD FIG. 4 illustrates the growth inhibitory activity of **NKEF** C against Jurkat human T-cell leukemia cells.

DRWD FIG. 5 illustrates the effect of **NKEF** C on VSV lytic infection.

DETD . . . leader sequence such that the mature protein comprises 241 amino acids. The protein exhibits the highest degree of homology to **NKEF** B expressed from NK-sensitive erythroleukemic cell line K 562, as shown in Sauri, H., et al. with 68.182% identity and. . .

DETD Fragments of the full length **NKEF** C gene may be used as a hybridization probe for a CDNA library to isolate the full length gene and to isolate other genes which have a high sequence similarity to the **NKEF** C gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for. . . identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete **NKEF** C gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the **NKEF** C gene by using the known DNA sequence to synthesize an oligonucleotide probe.

labeled oligonucleotides having a sequence complementary to. . .

DETD The present invention further relates to an **NKEF** C polypeptide which has the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or which has the amino. . .

DETD The **NKEF** C polypeptide of the present invention has been shown to significantly augment NK cell-mediated cytotoxicity when added at the initiation of cytotoxicity assays and **NKEF**, accordingly, may be employed to regulate NK function.

DETD The **NKEF** C polypeptide may be employed to enhance NK activity and therefore deter cancer development in the body. The **NKEF** C polypeptide may also be employed for immunoregulation of NK activity and may be important for cells in coping with. . .

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent inflammation.

DETD The **NKEF** C polypeptide of the present invention may also be employed to prevent NK-κB activity and prevent viral transcription and therefore. . . and therefore induce viral transcription. Accordingly, Human immunodeficiency virus type 1 (HIV-1) and HDLV-1 may also be treated with the **NKEF** C polypeptide of the present invention.

DETD This invention provides a method for identification of the receptor for the **NKEF** C polypeptide. The gene encoding the receptor can be identified by numerous methods known to those of skill in the. . . 1(2), Chapter 5, (1991)). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the **NKEF** C polypeptide, and a CDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the **NKEF** C polypeptide. Transfected cells which are grown on glass slides are exposed to labeled **NKEF** C polypeptide. The **NKEF** C polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a. . .

DETD . . . compounds to identify those which bind to and activate and those which bind to and inhibit the receptor for the **NKEF** C polypeptides. As an example, a mammalian cell or membrane preparation expressing the **NKEF** C receptor is incubated with a labeled compound to be tested. The compound may be labeled by a variety of. . . means known in the art, for example, by radioactivity. The ability of the compound to bind to and activate the **NKEF** C receptor could then be measured by the response of a known second messenger system. Such second messenger systems include,. . . are not limited to, CAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis. For instance, an effective agonist binds to the **NKEF** C receptor and elicits a second messenger response while an effective antagonist binds to the receptor but does not elicit. . .

DETD . . . oligopeptide, which binds to the polypeptide. Alternatively, a potential antagonist may be a closely related protein which binds to the **NKEF** C receptor, however, they are inactive forms of the polypeptide and thereby prevent the action of **NKEF** C since receptor sites are occupied.

DETD . . . et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of **NKEF** C. The antisense RNA oligonucleotide hybridizes to the MRNA in vivo and blocks translation of the mRNA molecule into **NKEF** C polypeptide (Antisense--Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)).. . . be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of **NKEF** C.

DETD The **NKEF** C polypeptides and agonists and antagonists which are polypeptides may also be employed in accordance with the present invention by. . .

DETD This invention is also related to the use of the **NKEF** C gene as a diagnostic. Detection of a mutated form of **NKEF** C will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of **NKEF** C for example, tumors and viral infections.

DETD Individuals carrying mutations in the human **NKEF** C gene may be detected at the DNA level by a variety of techniques. Nucleic acids for

diagnosis may be. . . cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding **NKEF** C can be used to identify and analyze **NKEF** C mutations, For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled **NKEF** C RNA or alternatively, radiolabeled **NKEF** C antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences. . .

DETD The present invention also relates to a diagnostic assay for detecting altered levels of **NKEF** C protein in various tissues since over-expression compared to normal control tissue samples can detect the presence of a tumor or viral infection. Assays used to detect levels of **NKEF** C protein in a sample derived from a host are well-known to those of skill in the art and include. . . assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the **NKEF** C antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter. . . serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any **NKEF** C proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked. . . peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to **NKEF**. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of **NKEF** C protein present in a given volume of patient sample when compared against a standard curve.

DETD A competition assay may be employed wherein antibodies specific to **NKEF** C are attached to a solid support and labeled **NKEF** C and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of **NKEF** C in the sample.

DETD Bacterial Expression and Purification of Soluble **NKEF**

DETD The DNA sequence encoding **NKEF**, ATCC #97157, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the **NKEF** C protein and the vector sequences 3' to **NKEF** C. Additional nucleotides corresponding to **NKEF** C were added to the 5' and 3' sequences respectively. The 5' oligonucleotide primers used for the full length sequence. . . with His-tag has the sequence 5' GCGCGGATCC ATGGAGGCGCTGCCCTGCT 3' (SEQ ID NO:3) contains a BamHI restriction enzyme site followed by **NKEF** C coding sequence starting from the presumed terminal amino acid of the processed protein and without the His-tag 5' CGCCCATGGAGGCGCTGCCCCTG 3' (SEQ ID NO:4) and contains a NcoI site. The 5' primer used for the **NKEF** C sequence without the leader sequence and without the His-tag is 5° CGCCCATGGCTGG AGCTGTGCAGGG 3' (SEQ ID NO:5) and has. . . used were as follows: 5' CGCGTCTAGATCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:6) which contains complementary sequences to an XbaI site followed by **NKEF** C coding sequence; and 5' CGCGTCTAGA TCAATTCAGTTTATCGAAATACTTCAGC 3' (SEQ ID NO:6). The restriction enzyme sites correspond to the restriction enzyme. . . then harvested by centrifugation. The cell pellet was solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized **NKEF** C was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). **NKEF** C was eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted. . .

DETD Cloning and Expression of **NKEF** C Using the Baculovirus Expression System

DETD The DNA sequence encoding the full length **NKEF** C protein, ATCC #97157, was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

DETD . . . signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987) and nucleotides of the

CGCGGATCCTCAATTCAGTTTATCGAAATAC 3' (SEQ ID NO:9) and contains the cleavage site for the restriction endonuclease BamHI and nucleotides complementary to the 3' non-translated sequence of the **NKEF** C gene.

DETD The vectors pA2-GP and pA2 (modifications of pVL941 vector, discussed below) are used for the expression of the **NKEF** C protein using the baculovirus expression system (for review see: Summers, M. D. and Smith, G. E. 1987, A manual. . .

DETD . . . T4 DNA ligase. E. coli HB101 cells were then transformed and bacteria identified that contained the plasmid (pBacNKEF) with the **NKEF** C gene using the enzyme BamHI. The sequence of the cloned fragment was confirmed by DNA sequencing.

DETD Sf9 cells were grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells were infected with the recombinant baculovirus V-**NKEF** C at a multiplicity of infection (MOI) of 2. Six hours later the medium was removed and replaced with SF900. . .

DETD Expression of Recombinant **NKEF** C in COS Cells

DETD The expression of plasmid, **NKEF** C HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3). . . 4) CMV promoter followed by a polylinker region, an SV40 intron and polyadenylation site. A DNA fragment encoding the entire **NKEF** C precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of. . .

DETD The DNA sequence encoding **NKEF**, ATCC #97157, was constructed by PCR on the original EST cloned using two primers: the 5' primer 5' GCGCGGATCCACCATGGAGGCGCTG 3' (SEQ ID NO:12) contains a BamHI site followed by 12 nucleotides of **NKEF** C coding sequence starting from the initiation codon; the 3' sequence 5' GCGCTCTAGATCAAGCGTAGTCTGGACGTCGT ATGGGTAATTCAGTTTATC 3' (SEQ ID NO:13) contains complementary sequences to an XbaI site, translation stop codon, HA tag and the last 12 nucleotides of the **NKEF** C coding sequence (not including the stop codon). Therefore, the PCR product contains a BamRI site, **NKEF** C coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag,. . . isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant **NKEF**, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the **NKEF** C HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring. . .

DETD Growth Inhibitory Activity of **NKEF** C Against Human Leukemia Cells

DETD Two-fold serial dilution of purified **NKEF** C starting from 100 ng/ml were made in RPMI 1640 medium with 0.5% FBS. HL60 or Jurkat cells were harvested. . .

DETD **Antiviral** Activity of **NKEF** C Against Vesicular Stomatitis Virus (VSV)

DETD The cytopathic effect reduction (CPER) assay is employed to measure the protective effect of **NKEF** C on the infection and cytopathic process of vesicular stomatitis virus (VSV) to normal human dermal fibroblasts (NHDF) from foreskin (Clonetics). In this experiment we performed serial dilution of **NKEF** C at a 1:2 ratio and extended the dilution starting from 3 μg/ml to 6 ng/ml final concentration. The positive. . . 100 μl. In addition, we maintained a negative (untreated) mock control. Semi-purified (.about.70%) protein isolated from E. Coli expressing the **NKEF** C protein was employed in this study. The NHDF cells were seeded at 2×10^4 /well and incubated overnight to reach. . .
scored for CPE by estimating the percentage of cells surviving on the microtiter plate. The figure demonstrates a mean effective **NKEF** C concentration equal to .about.100 ng/ml.

. . . amino acids 31-271 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide. . .

. . . amino acids of 171 of SEQ ID NO:2; (d) a polynucleotide encoding full length human Natural Killer Cell Enhancing Factor C (**NKEF** C) having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (e) a polynucleotide encoding mature **NKEF** C having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97157; (f) a polynucleotide. . .

=> d his

(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

FILE 'USPATFULL' ENTERED AT 12:15:57 ON 02 MAY 2004

FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
E LYNN RALF GEIBEN/IN
L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
E LYNN R G/IN
L4 2 S E3

FILE 'MEDLINE' ENTERED AT 12:21:25 ON 02 MAY 2004
E LYNN R G/AU
E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
E WALKER B D/AU
L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L10 101 S L9 AND (CD8? OR CYTOTOXIC OR ANTIVIRAL? OR SUPPRESSOR? OR SOL

FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
E GEIBEN-LYNN R/IN
L11 1 S E2
L12 526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13 120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14 120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15 11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16 10 S L15 NOT (L1 OR L11)
L17 109 S L13 NOT L15
L18 54 S L17 AND AY<2002

FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19 535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20 3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21 3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22 2 S L21 NOT L20
E BUTTERFIELD L H/AU
L23 23 S E3
E SHAU H/AU
L24 65 S E3-E5
L25 1 S L19 AND (ANTIVIR?)

FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
L26 105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L27 3 S L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR
L28 2 S L27 NOT L4

FILE 'IFIPAT' ENTERED AT 12:59:18 ON 02 MAY 2004

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L29           3 S (US 2001-952843)/PN,APPS

     FILE 'USPATFULL' ENTERED AT 12:59:18 ON 02 MAY 2004
L30           3 S (US 2001-952843)/PN,APPS

     FILE 'USPATFULL' ENTERED AT 12:59:38 ON 02 MAY 2004

     FILE 'USPATFULL' ENTERED AT 13:01:29 ON 02 MAY 2004
               E GENTZ R/IN
L31         142 S E3-E7
L32          56 S L31 AND (NKEF? OR ANTIVIRAL? OR NATURAL KILLER ENHANCING FACT
L33          56 S L32 AND (ANTIVIR?)
L34          39 S L33 AND AY<2002

=> e browning l/ain
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The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
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E3            0 --> BROWNING L/IN
E4            1     BROWNING LARRY E/IN
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E6            1     BROWNING LAURA A/IN
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E12           1     BROWNING LUTHER C/IN

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E12           1     WAN WENSHUAI/IN

=> s e11
L35           1 "WAN WEN HUI"/IN

=> d l35,cbib,ab

L35  ANSWER 1 OF 1  USPATFULL on STN
90:25687 Method for preparing multiple tissue samples for microscopic
    investigation and testing.
    Furmanski, Philip, Littleton, CO, United States
    Fortuna, Michael B., Denver, CO, United States
    Wan, Wen-Hui, Lakewood, CO, United States
    AMC Cancer Research Center, Denver, CO, United States (U.S. corporation)
    US 4914022 19900403
    APPLICATION: US 1987-110818  19871021 (7)
    DOCUMENT TYPE: Utility; Granted.
AB     A flexible, efficient and rapid method is shown whereby many different
       tissues samples may be stained or reacted and examined simultaneously,
       on one standard glass slide. This method is based on the preparation of
```

as histology blocks. The paraffin embedded tissue cores are inserted into a casing cut from an ordinary drinking straw, or similar holding device, mounted in a paraffin block and sectioned.

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=> e e12
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E5            3     WAN WINNIE H/IN
E6            2     WAN WINSTON L/IN
E7            2     WAN XIANG/IN
E8            1     WAN XIAO YANG/IN
E9            2     WAN XINGSHENG/IN
E10           1     WAN XUDONG/IN
E11           1     WAN YE K/IN
E12           1     WAN YE NU/IN

=> s e5
L36           3 "WAN WINNIE H"/IN

=> d l36,ti,1-3

L36  ANSWER 1 OF 3  USPATFULL on STN
TI     Compositions comprising mixtures of therapeutic proteins and methods of
       producing the same

L36  ANSWER 2 OF 3  USPATFULL on STN
TI     Compositions comprising mixtures of therapeutic proteins and methods of
       producing the same

L36  ANSWER 3 OF 3  USPATFULL on STN
TI     Compositions comprising mixtures of therapeutic proteins and methods of
       producing the same


=> d l36,cbib,ab,1-3

L36  ANSWER 1 OF 3  USPATFULL on STN
2003:187376 Compositions comprising mixtures of therapeutic proteins and
    methods of producing the same.
    Lau, Allan S., Pok Fu Lam, HONG KONG
    Wan, Winnie H., Woodside, CA, UNITED STATES
    Browning, Laura, Brentwood, CA, UNITED STATES
    Ossina, Natalya, Albany, CA, UNITED STATES
    US 2003129162 A1 20030710
    APPLICATION: US 2002-241970  A1  20020911 (10)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Human cytokine mixtures produced by cytokine regulatory
       factor-overexpressing cells and methods of production are disclosed. The
       mixtures are prepared by culturing human cytokine-producing cells under
       conditions of cytokine regulatory factor overexpression, treating the
       cells to induce cytokine production, and isolating the mixtures of
       cytokines produced by the cells. Preferred compositions, for use in
       treating viral infection or cancer, include a mixture of human
       interferon γ and either human interferon α or human
       interferon β, in a mole ratio of between 2:1 to 1:100 interferon
       γ to interferon α or human interferon β.

L36  ANSWER 2 OF 3  USPATFULL on STN
2002:272437 Compositions comprising mixtures of therapeutic proteins and
    methods of producing the same.
    Lau, Allan S., Pok Fu Lam, HONG KONG
```

[illegible], Woodside, CA, UNITED STATES
Browning, Laura, Brentwood, CA, UNITED STATES
Ossina, Natalya, Albany, CA, UNITED STATES
US 2002150552 A1 20021017
APPLICATION: US 2001-952843 A1 20010911 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human cytokine mixtures produced by cytokine regulatory factor-overexpressing cells and methods of production are disclosed. The mixtures are prepared by culturing human cytokine-producing cells under conditions of cytokine regulatory factor overexpression, treating the cells to induce cytokine production, and isolating the mixtures of cytokines produced by the cells.

L36 ANSWER 3 OF 3 USPATFULL on STN
2002:272426 Compositions comprising mixtures of therapeutic proteins and methods of producing the same.
Lau, Allan S., Pok Fu Lam, HONG KONG
Wan, Winnie H., Woodside, CA, UNITED STATES
Browning, Laura, Brentwood, CA, UNITED STATES
Ossina, Natalya, Albany, CA, UNITED STATES
Gene Trol Biotherapeutics, Inc. (non-U.S. corporation)
US 2002150541 A1 20021017
APPLICATION: US 2002-105100 A1 20020321 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human cytokine mixtures produced by cytokine regulatory factor-overexpressing cells and methods of production are disclosed. The mixtures are prepared by culturing human cytokine-producing cells under conditions of cytokine regulatory factor overexpression, treating the cells to induce cytokine production, and isolating the mixtures of cytokines produced by the cells. Exemplary compositions include mixtures of human interferon γ in combination with human interferon α and/or human interferon β, and mixtures of human interferon α and human interferon β. Also disclosed are therapeutic uses of the interferon compositions.

=> d his

(FILE 'HOME' ENTERED AT 12:15:32 ON 02 MAY 2004)

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FILE 'USPATFULL' ENTERED AT 12:17:09 ON 02 MAY 2004
E LYNN RALF GEIBEN/IN
L1 1 S E3
E WALKER BRUCE D/IN
L2 7 S E3
L3 6 S L2 NOT L1

FILE 'WPIDS' ENTERED AT 12:19:51 ON 02 MAY 2004
E LYNN R G/IN
L4 2 S E3

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E LYNN RALF G/AU
E GEIBEN-LYNN R/AU
E GEIBEN LYNN R/AU
L5 5 S E3 OR E4
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L6 155 S E3
L7 154 S L6 NOT L5
L8 0 S L7 AND (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FAC
L9 127 S L7 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN

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L10           101 S L9 AND (CD8? OR CYTOKINE OR ANTIVIRAL? OR SUPPRESSOR? OR DOZ

     FILE 'USPATFULL' ENTERED AT 12:34:48 ON 02 MAY 2004
                E GEIBEN-LYNN R/IN
L11           1 S E2
L12         526 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L13         120 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L14         120 S L13 AND (TREAT? OR THERAPEUT? OR ANTIVIR?)
L15          11 S L14 AND (PEROXIREDOX?/CLM OR NKEF?/CLM OR NATURAL KILLER ENHA
L16          10 S L15 NOT (L1 OR L11)
L17         109 S L13 NOT L15
L18          54 S L17 AND AY<2002

     FILE 'MEDLINE' ENTERED AT 12:41:56 ON 02 MAY 2004
L19         535 S (PEROXIREDOX? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR? OR
L20           3 S L19 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L21           3 S L19 AND (SIV OR SIMIAN IMMUNODEFICIENCY VIRUS OR LENTIVIR? OR
L22           2 S L21 NOT L20
                E BUTTERFIELD L H/AU
L23          23 S E3
                E SHAU H/AU
L24          65 S E3-E5
L25           1 S L19 AND (ANTIVIR?)

     FILE 'WPIDS' ENTERED AT 12:55:24 ON 02 MAY 2004
L26         105 S (PEROXIREDOXIN? OR NKEF? OR NATURAL KILLER ENHANCING FACTOR?
L27           3 S L26 AND (ANTIVIR? OR HIV? OR HUMAN IMMUNODEFICIENCY VIRUS OR
L28           2 S L27 NOT L4

     FILE 'IFIPAT' ENTERED AT 12:59:18 ON 02 MAY 2004
L29           3 S (US 2001-952843)/PN,APPS AND AB/FA

     FILE 'USPATFULL' ENTERED AT 12:59:18 ON 02 MAY 2004
L30           3 S (US 2001-952843)/PN,APPS

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     FILE 'USPATFULL' ENTERED AT 13:01:29 ON 02 MAY 2004
                E GENTZ R/IN
L31         142 S E3-E7
L32          56 S L31 AND (NKEF? OR ANTIVIRAL? OR NATURAL KILLER ENHANCING FACT
L33          56 S L32 AND (ANTIVIR?)
L34          39 S L33 AND AY<2002
                E BROWNING L/IN
                E WAN W H/IN
L35           1 S E11
                E E12
L36           3 S E5

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
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